

**EFFECT OF ARTHROSCOPIC LAVAGE AND REPEATED
THROUGH-AND-THROUGH JOINT LAVAGE ON SYSTEMIC AND
SYNOVIAL SERUM AMYLOID A CONCENTRATIONS; AS WELL AS
TOTAL PROTEIN CONCENTRATION, NUCLEATED CELL COUNT
AND PERCENTAGE OF NEUTROPHILS IN SYNOVIAL FLUID
FROM HEALTHY EQUINE JOINTS**

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
Saskatoon

By

ANDRES FERNANDO SANCHEZ TERAN

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

DISCLAIMER

Reference in this thesis to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise; does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Head of the Department of Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, Saskatchewan S7N 5B4
Canada

OR

Dean
College of Graduate Studies and Research
University of Saskatchewan
107 Administration Place
Saskatoon, Saskatchewan S7N 5A2
Canada

ABSTRACT

EFFECT OF ARTHROSCOPIC LAVAGE AND REPEATED THROUGH-AND-THROUGH JOINT LAVAGE ON SYSTEMIC AND SYNOVIAL SERUM AMYLOID A CONCENTRATIONS; AS WELL AS TOTAL PROTEIN CONCENTRATION, NUCLEATED CELL COUNT AND PERCENTAGE OF NEUTROPHILS IN SYNOVIAL FLUID FROM HEALTHY EQUINE JOINTS

Andrés F. Sánchez-Terán

University of Saskatchewan, 2015

Advisors:

Dr. José L. Bracamonte

Dr. Luis M. Rubio-Martínez

This research evaluated serum amyloid A (SAA) concentration in synovial fluid of healthy horses as a potential marker for use in the diagnosis and monitoring of horses with septic arthritis. The first study evaluated the effect of arthroscopic lavage of healthy joints on concentrations of systemic and synovial SAA; as well as total protein concentration, nucleated cell count and percentage of neutrophils in synovial fluid. The second study, evaluated the effect of repeated through-and-through joint lavage on SAA in systemic blood and SAA, total protein, nucleated cell count and percentage of neutrophils in synovial fluid from healthy joints.

In the first study, middle carpal joints of 6 horses were randomly assigned to one of the following treatments 1) arthrocentesis (controls) or 2) arthroscopic lavage. A washout period of 30 days was allowed in between treatments. Synovial fluid and blood samples were collected at 0, 24, 48, 72, 96 and 120 h. Measurements included SAA in blood and synovial fluid, and total protein, nucleated cell count and percentages of neutrophils in synovial fluid.

In the second study, one tarsocrural joint was randomly assigned to receive repeated through-and-through joint lavage at 0, 48 and 96 h in 6 horses. Synovial fluid and blood samples were collected at 0, 24, 48, 72, 96 and 120 h. Measurements included SAA in blood and synovial fluid, and total protein, nucleated cell count and percentages of neutrophils in synovial fluid. For this study, synovial fluid samples collected at time 0 were considered as control values.

After arthroscopic lavage and repeated through-and-through joint lavage, systemic and synovial SAA did not increase from baseline values (except for systemic SAA at 24h after arthroscopic lavage and in controls). Total protein values were significantly increased at all time points after arthroscopic and through-and-through joint lavages (except at 96h on both lavage procedures) but not in controls. With both lavage procedures, nucleated cell count significantly increased from baseline values at all time points (except at 96h after through-and-through joint lavage). Percentage of neutrophils was significantly increased after arthroscopic lavage at all time points and only at 24h in controls; however, the percentages of neutrophils were not significantly increased after repeated through-and-through joint lavage.

Synovial SAA was not affected by arthroscopic or repeated through-and-through joint lavage; however, synovial total protein and nucleated cell counts were significantly increased. Synovial SAA may be a valuable inflammatory marker that is not affected by procedures as arthroscopic or repeated through-and-through joint lavage in horses. Further validation of synovial SAA as a marker for evaluating the progression of septic joints while treatment is installed is warranted.

AKNOWLEDGEMENTS

I would like to thank Dr. Joe Bracamonte, my supervisor through my Posgraduate degree and my Residency in Large Animal Surgery; as well as Dr. Luis Rubio-Martinez as my promoters and friends, for all their patience, personal support and the effort they put into helping me finish this thesis on time.

I would also like to thank Dr. Steven Hendrick for the statistical analysis performed as well as his support through my degree.

Thanks to Lea Riddell and Monica Schott, my summer students. Thank you to all the team at the Large Animal Surgery Department; especially Linda Hueller and Leanne Martin for their help when performing the studies.

DEDICATIONS

To God, to who owns my life, past, present and future. To whom I am grateful for all my achievements, has guided me along all my steps, carry me through all circumstances and has brought me to places I never thought about. Thank you with all my heart.

I dedicate my work especially to my parents, to whom I owe my life and my education. Without their support and love I could not have reached my dreams. To my grandparents, who showed me the joys of life and taught me with their incredible wisdom.

To Dianne Peacock and Sara Peacock, my Canadian family, for the support, love, care, knowledge and advice they have shared through my stay in Saskatoon.

TABLE OF CONTENTS

| | |
|--|------|
| PERMISSION TO USE | i |
| DISCLAIMER | i |
| ABSTRACT | ii |
| ACKNOWLEDGEMENTS | iv |
| DEDICATIONS | v |
| TABLE OF CONTENTS | vi |
| LIST OF TABLES | ix |
| LIST OF FIGURES | x |
| LIST OF ABBREVIATIONS | xiii |
| CHAPTER 1: INTRODUCTION | 1 |
| 1.1 Objectives and Hypotheses | 3 |
| CHAPTER 2: LITERATURE REVIEW | 4 |
| 2.1 Introduction..... | 4 |
| 2.2 Septic synovitis in the horse..... | 4 |
| 2.2.1 Prognosis and factors associated with survival and return to previous performance .. | 4 |
| 2.2.2 Causes of septic synovitis and common bacterial isolates | 6 |
| 2.2.3 Inflammatory response of the synovial structure to bacteria..... | 7 |
| 2.2.4 Diagnosis and monitoring of septic synovitis..... | 9 |

| | |
|--|-----------|
| 2.3 Treatment options for septic synovitis and their effects on common synovial inflammatory markers..... | 11 |
| 2.3.1 Arthroscopic lavage..... | 13 |
| 2.3.2 Through-and-through joint lavage | 15 |
| 2.3.3 Antimicrobial administration | 16 |
| 2.4 Biology of serum amyloid A..... | 18 |
| 2.4.1 Molecular architecture, genes expression and isoforms..... | 19 |
| 2.4.2 Transport through the bloodstream and metabolism of serum amyloid A..... | 21 |
| 2.5 Serum amyloid A biological functions | 21 |
| 2.5.1 Regulatory mechanisms of serum amyloid A..... | 22 |
| 2.5.2 Serum amyloid A role in immunity | 22 |
| 2.5.3 Anti-inflammatory properties of serum amyloid A..... | 23 |
| 2.6 Serum amyloid A as a marker of systemic inflammation in horses..... | 23 |
| 2.7 Serum amyloid A in synovial fluid..... | 24 |
| 2.7.1 The role of serum amyloid A in rheumatoid arthritis | 25 |
| 2.7.2 The role of serum amyloid A in osteoarthritis..... | 25 |
| 2.7.3 Serum Amyloid A in synovial fluid from horses | 26 |
| 2.8 Methods for determination of equine serum amyloid A concentration..... | 28 |
| 2.9 Other synovial fluid markers used in the diagnosis of septic arthritis | 29 |
| 2.10 Conclusions | 30 |

CHAPTER 3: EFFECT OF ARTHROSCOPIC LAVAGE ON SYNOVIAL FLUID CONCENTRATIONS OF SERUM AMYLOID A, TOTAL PROTEIN AND NUCLEATED CELL COUNT IN HEALTHY HORSES

| | |
|--|-----------|
| 3.1 Abstract..... | 32 |
| 3.2 Introduction | 33 |
| 3.3 Material and methods | 34 |
| 3.3.1 Procedures | 35 |
| 3.3.2 Postoperative sampling and monitoring | 36 |
| 3.3.3 Sample analysis | 37 |
| 3.3.4 Statistical analysis | 38 |

| | |
|---|--------|
| 3.4 Results..... | 38 |
| 3.4.1 Protein concentrations in synovial fluid | 39 |
| 3.4.2 Nucleated cell counts in synovial fluid | 40 |
| 3.4.3 Percentage of neutrophils in synovial fluid | 40 |
| 3.4.4 Serum amyloid A in synovial fluid | 41 |
| 3.4.5 Systemic serum amyloid A | 41 |
| 3.5 Discussion..... | 42 |
| CHAPTER 4: EFFECT OF REPEATED THROUGH-AND-THROUGH NEEDLE LAVAGE ON SERUM AMYLOID A IN SYNOVIAL FLUID FROM HEALTHY JOINTS | 51 |
| 4.1 Abstract..... | 52 |
| 4.2 Introduction | 53 |
| 4.3 Material and methods | 54 |
| 4.3.1 Sample analysis | 55 |
| 4.3.2 Statistical analysis | 56 |
| 4.4 Results..... | 56 |
| 4.5 Discussion..... | 57 |
| CHAPTER 5: GENERAL DISCUSSION | 65 |
| CHAPTER 6: CONCLUSIONS AND FUTURE STUDIES | 67 |
| CHAPTER 7: MASTER REFERENCE LIST | 70 |
| CHAPTER 8: APPENDICES AND SUPPORTING DOCUMENTATION | 89 |
| 8.1 Data for Chapter 4 | 89 |
| 8.2 Data for Chapter 5 | 93 |
| 8.3 Animal Care Certificate of Approval for experimental study of Chapter 4..... | 96 |
| 8.4 Animal Care Certificate of Approval for experimental study of Chapter 5..... | 97 |
| CHAPTER 9: Vita..... | 98 |

LIST OF TABLES

| | |
|---|----|
| Table A1. Raw data after arthroscopic lavage..... | 89 |
| Table A2. Summary statistics for controls: p25, p50 (median) and p75; by time | 91 |
| Table A3. Summary statistics for arthroscopic lavage group: p25, p50 (median) and p75; by time..... | 92 |
| Table A4. Raw data after repeated through-and-through joint lavage..... | 93 |
| Table A5. Raw data blood contamination and needle repositioning | 94 |
| Table A6. Summary statistics after repeated through-and-through joint lavage: p25, p50 (median) and p75; by time..... | 95 |

LIST OF FIGURES

- Figure 1.** Box plot of median (circles) total protein concentrations (g/L) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference (p<0.05) from baseline. ⁺Significant difference (p<0.05) between treatments. 46
- Figure 2.** Box plot of median (circles) nucleated cell counts (x10⁹ cell/L) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference (p<0.05) from baseline. ⁺Significant difference (p<0.05) between treatments. 47
- Figure 3.** Box plot of median (circles) percentage of neutrophils (%) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference (p<0.05) from baseline. ⁺Significant difference (p<0.05) between treatments. 48
- Figure 4.** Box plot of median (circles) SAA concentrations (mg/L) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant

difference ($p<0.05$) from baseline. ⁺Significant difference ($p<0.05$) between treatments..... 49

Figure 5. Box plot of median (circles) systemic SAA concentrations (mg/L) from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference ($p<0.05$) from baseline. ⁺Significant difference ($p<0.05$) between treatments. 50

Figure 6. Box plot of median (circles) total protein concentrations (g/L) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ^xSignificant difference ($p<0.05$) from baseline. 60

Figure 7. Box plot of median (circles) nucleated cell counts ($\times 10^9$ cells/L) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ^xSignificant difference ($p<0.05$) from baseline. 61

Figure 8. Box plot of median (circles) neutrophils (%) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ^xSignificant difference ($p<0.05$) from baseline. 62

Figure 9. Box plot of median (circles) serum amyloid A (mg/L) in synovial fluid after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers

correspond to maximum and minimum values. ^XSignificant difference ($p<0.05$)
 from baseline. 63

Figure 10. Box plot of median (circles) systemic serum amyloid A (mg/L) after
 repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of
 box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond
 to maximum and minimum values. ^XSignificant difference ($p<0.05$) from
 baseline. 64

LIST OF ABBREVIATIONS

| | |
|------------------|---|
| APPs | acute phase proteins |
| APR | acute phase response |
| CBC | complete blood work |
| cDNA | complementary deoxyribonucleic acid |
| CO ₂ | carbon dioxide |
| CRP | C-reactive protein |
| DMSO | dimethylsulfoxide |
| ELISA | enzyme-linked immunosorbent assay |
| FPRL1 | formyl peptide receptor-like 1 |
| g/L | grams per liter |
| GM-CSF | growth-promoting granulocyte-macrophage colony-stimulating factor |
| h | hour |
| HDL ₃ | fraction 3 of high-density lipoprotein |
| IL-1 | interleukin 1 |
| IL-10 | interleukin 10 |
| IL-6 | interleukin 6 |
| IORLP | intraosseous regional limb perfusion |
| IVRLP | intravenous regional limb perfusion |
| L | liter |
| LDL | low-density lipoprotein |
| LOQ | lower limit of quantification |
| LPS | lipopolysaccharides |
| LRS | lactated Ringer's solution |
| mg/L | milligrams per liter |
| MIC | minimal inhibitory concentration |
| MMPs | matrix metalloproteinases |
| mRNA | messenger ribonucleic acid |

| | |
|---------------|--|
| NCC | nucleated cell count |
| NSAIDs | non-steroidal anti-inflammatory drugs |
| ORF | open reading frame |
| RAGE | receptor for the advanced glycation end products |
| SAA | serum amyloid A |
| SSGD | serum and synovial fluid glucose difference |
| TLR4 | toll-like receptor 4 |
| TNF- α | tumor necrosis factor α |
| VLDL | very low density lipoprotein |

CHAPTER 1: INTRODUCTION

Septic synovitis is a severe disease that affects horses of all ages and can be life-threatening. Over the last three decades, it has been a major topic of research because of the severe consequences it may have on affected horses. Accurate and prompt diagnosis followed by aggressive treatment increase the possibilities for survival and return to sporting activities.

The main tools for diagnosing and monitoring treatment of synovial sepsis have typically been based on the use of inflammatory markers and bacterial culture from synovial fluid samples. Bacteria are not always isolated from synovial fluid or evident on cytology and consequently the specificity of these tests is low.^{11,24} Therefore, diagnosis and monitoring are based on clinical examination and analysis of synovial fluid. The common inflammatory markers analysed in synovial fluid include total protein concentration, nucleated cell count and the percentage of neutrophils. Although these inflammatory markers are widely used, they have shown limited accuracy as they can increase from any insult to the synovial structure, and not just from sepsis.

Treatment of infection in a synovial structure is based on two major principles: eradication of infective organisms and rehabilitation of the synovial structure. The initial cleaning of the infected cavity is performed by active debridement and thorough lavage with sterile solution reducing the amount of bacteria as well as the inflammatory mediators present within the cavity. This is accompanied by the administration of systemic and regional antimicrobials to reduce further bacterial presence and multiplication within the joint. The preferred approach to lavage and debride the affected cavity is by the use of arthroscopy. Another option to provide lavage of a synovial structure is through needles placed on different pouches of the synovial cavity (through-and-through joint lavage). These procedures and the intra-synovial administration of antimicrobials increase the commonly used synovial inflammatory markers to values similar to those associated with synovial sepsis.^{78,79,94} Therefore, these inflammatory markers are not reliable especially after the joint has been treated; exposing the need to find new inflammatory markers that are not affected by the procedures used to resolve synovial sepsis.

Serum amyloid A (SAA) in synovial fluid is an inflammatory marker that has promising characteristics as a more specific marker for sepsis. While SAA has been shown to increase in

synovial fluid to high concentrations during septic synovitis, its values were not modified by either arthrocentesis or intra-articular administration of amikacin.⁹⁴ However, further evaluation of this marker after high volume fluid lavage by either arthroscopy or repeated through-and-through joint lavage is needed before clinical use is recommended as a tool for monitoring treatment of synovial sepsis. If SAA concentration in synovial fluid is not modified after arthroscopic lavage or repeated through-and-through joint lavage in healthy horses, postoperative measurements of SAA in synovial fluid could be useful as a more specific marker for monitoring the response of treatment in septic synovitis. Advantages of validation of an specific marker for septic synovitis that is not affected by these therapeutic procedures not only will provide a prompt and accurate identification of treatment failure or success; but also might help in reducing hospitalization time, drugs administered, as well as serve as a prognostic indicator for survival or return to athletic performance.

1.1 Objectives and Hypotheses

This work includes 2 studies:

The first study was designed to evaluate the effect of arthroscopic lavage on systemic and synovial fluid SAA concentrations, as well as the effects on total protein concentration, nucleated cell count and percentage of neutrophils in synovial fluid in healthy equine middle carpal joints. It was hypothesized that:

- 1) Total protein concentration, nucleated cell counts and percentage of neutrophils in synovial fluid would significantly increase after arthroscopic lavage.
- 2) Systemic and synovial fluid SAA concentrations would not increase after arthroscopic lavage.

The second study was designed to evaluate the effect of repeated through-and-through joint lavage on systemic and synovial fluid SAA concentrations, and total protein concentrations, nucleated cell count and percentage of neutrophils in synovial fluid in healthy equine tarsocrural joints. It was hypothesized that:

- 1) Total protein concentration, nucleated cell counts and percentage of neutrophils in synovial fluid would significantly increase after repeated through-and-through joint lavage.
- 2) Systemic and synovial fluid SAA concentrations would not increase after repeated through-and-through joint lavage.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The understanding of septic synovitis as a common disease, how the synovial cavity reacts to infection, as well as the different treatment options and their effect on the common synovial inflammatory markers are exposed in the following literature review. Furthermore, SAA current research on its use as an inflammatory marker in horses as well as its recognized systemic and local (within the joint) functions are summarized to improve the understanding of this promising inflammatory marker for septic synovitis.

2.2 Septic synovitis in the horse

Septic synovitis is defined as a “purulent invasion of a synovial cavity by an infectious agent, which produces synovitis”.¹ Infection of a synovial cavity is one of the most feared and severe diseases affecting horses of all ages. Clinical signs include synovial effusion, heat over the affected structure, and frequently is associated with severe lameness.² Causes for horses acquiring infection of a synovial structure are varied and can be due to a penetrating wound,^{3,4} hematogenous spread,⁵ and iatrogenic by intra-synovial injections or surgical intervention.⁶⁻⁸ The inflamed synovial cavity produces a strong inflammatory response to overcome the infection, which can produce damage to the cartilage leading to osteoarthritis. Treatment is prolonged and prognosis is not always favorable. It can be life-threatening as well as reduce the performance and future competitiveness of sport horses. Consequences include chronic pain, joint stiffness and contra-lateral limb laminitis amongst others.^{9,10} Research in the field of diagnosis and treatment of equine synovial infection has been quite extensive over the last decades.⁶

2.2.1 Prognosis and factors associated with survival and return to previous performance

Survival rates after septic synovitis vary according to the age group affected. Discharge rates from hospital after intensive treatment are lower in foals (45%) than in adult horses (85%) due to concurrent conditions such as osteomyelitis, multiple joint involvement,

hypogammaglobulinaemia and multisystemic disease.^{4,11-13} Survival rates for horses having septic tenosynovitis have also been reported where 91% of treated horses survived to discharge.

The future career of horses affected by synovial sepsis has also been investigated and varies with age and whether infection is present in a joint or a tendon sheath. Only 48% of Thoroughbred foals that survived to discharge (45%) after being treated for septic arthritis started in at least one race in their career, which is less than the average of 66.2% of healthy foals that race.¹⁴ Considering only adult horses being discharged from hospital, 56% to 81% of these horses were able to return to racing after synovial sepsis.^{2,4,11} In another report, 11 of 12 horses returned to their previous level of performance.⁸ Furthermore, only 69% of the horses surviving to discharge after septic tenosynovitis, returned to their previous athletic use.^{15,16} However, another study reported a lower rate of 56% of horses with septic tenosynovitis returning to previous use.¹⁷

Different factors have been reported to be associated with survival. These include time from injury to treatment,^{16,18,19} positive bacterial cultures,² presence of bone or tendon pathology,^{4,12,16,18,20} identification of inflammatory pannus^{4,18,19} or osteochondral fragments,¹⁸ elevated nucleated cell count (NCC),^{12,21} increased matrix metalloproteinases (MMPs)²¹ and total protein,¹⁹ timing of general anesthesia and total number of surgeries performed.¹⁹ Also, iatrogenic infection after intra-articular injection with corticosteroids has been associated with poor prognosis.⁸

Early recognition and aggressive treatment of synovial sepsis has been associated with better prognosis for survival and return to athletic function than when treatment is delayed in adult horses.^{6,9} Horses treated within the first 24 h after contamination of a synovial structure (open joint injury) were less likely to develop infectious arthritis and more likely to return to normal performance than horses treated after 24 h.²² In a recent study on septic tarsocrural joints, early treatment resulted in complete recovery, while chronic cases had prolonged recovery without achieving soundness.²⁰ However, in some cases, aggressive treatment after several days from the initial joint contamination can also lead to satisfactory outcomes with horses returning to previous athletic function.^{2,4,8,12,14,15,19,21,23} Contradictory results have been also reported in regards to septic tenosynovitis. Three studies suggest that the time gap between injury and appropriate treatment had no impact in the outcome and might not be useful for formulating a prognosis.^{11,15,17} However, one study found that treatment initiated within 36 h after injury

carried a better prognosis for returning to athletic performance (89%) than when treatment was initiated after 36 h (40%).¹⁶ Furthermore, a study found horses being treated within 12 h after injury had a worse prognosis maybe due to the severity of the initial wound which was noticed sooner by owners and warranted immediate attention and euthanasia.¹⁶

Although bacteria are present during synovial sepsis, they are difficult to isolate^{11,24} and the absence of a positive bacterial culture does not indicate absence of synovial sepsis.^{24,25} One study reported that a positive culture had no effect on the likelihood of horses surviving to hospital discharge.¹¹ On the other hand, successful isolation of bacteria from an infected synovial structure was associated with a poorer prognosis for survival in a different study.² In the latter, only 74.6% of horses with synovial sepsis that yielded a positive culture survived to hospital discharge compared to 97.1% survival of horses with synovial sepsis that did not yield a positive culture.² In addition, culture of *Staphylococcus aureus* had a negative effect on long-term outcome⁸ and these horses were 29.5 times more likely to be euthanized than horses that yield a negative culture.² Horses that survived after *S. aureus* synovial infection had a reduced likelihood of returning to previous level of performance (30.8%).² In addition, horses that yielded another bacterial genus different from *S. aureus* were only 13.9 times more likely to be euthanized than horses that yielded a negative culture.²

The presence of tendon or bone involvement has also been associated with decreased survival and reduced athletic outcome.¹² Horses with septic tarsocrural joints with radiographic subchondral bone lysis and osteophyte formation were found to have longer convalescence and lower percentage returned to athletic activity compared to horses without radiographic abnormalities.²⁰ Bone or tendon pathology are not always noticed on initial assessment and repeated examinations during treatment are indicated in refractory cases.¹²

2.2.2 Causes of septic synovitis and common bacterial isolates

Causes for developing synovial sepsis vary with age of patient. Foals are more prompt to acquire septic arthritis during septicemia while in adult horses wounds are the most common cause for septic synovitis.^{2,20} Often, several joints are involved in foals and Gram-negative bacteria generally are the cause of septic polyarthritis and osteomyelitis.^{11,26} Obtaining positive bacterial cultures in foals with septic arthritis diagnosed based on cytological results, is

challenging and two studies have reported that only 32.5%¹¹ and 11%²⁶ of samples yielded a positive culture. Septic synovitis after a wound is commonly associated with the introduction of microorganisms along with foreign material,^{2,20} exacerbating the infection.^{11,17,18} After a wound involving a synovial structure, only 32.9% of synovial samples yielded a positive bacterial culture.² The synovial structure most commonly infected after a wound is the tarsocrural joint (22.4%) followed by the metacarpal/metatarsal-phalangeal joints (20.1%) and the digital flexor tendon sheath (18.7%).²

Commonly isolated microorganisms during septic synovitis include aerobes or facultative anaerobes. The most commonly cultured genus of bacteria is Enterobacteriaceae followed by *Streptococcus spp.* and *Staphylococcus spp.*²⁷ A variety of bacteria can be expected in cases of perforating wounds, while *Staphylococcus spp.* is more commonly isolated in infections after surgical intervention.^{8,11} When a cause for synovial sepsis in adult horses cannot be identified, it is defined as idiopathic synovitis. This entity is thought to have a hematogenous origin and Gram-positive bacteria are usually identified.¹¹

Iatrogenic contamination can occur after intrasynovial injection and after a surgical procedure involving a synovial structure.^{2,7} Sepsis following synoviocentesis and surgery is commonly related to *Staphylococcus aureus*. However other bacteria like *Streptococcus spp.*, Enterobacteriaceae, *Pseudomonas spp.* and *Klebsiella spp.* have been isolated.^{3,8,11} In a recent study, only 0.5% of joints developed septic arthritis as a complication after elective arthroscopy without perioperative antimicrobial therapy.⁷ In a large retrospective study where 2833 medical records were reviewed from horses receiving intra-articular injections without prophylactic antimicrobials, it was found that the incidences of septic arthritis after an intra-articular injection with any substance was 0.092%, after local anesthetic 0.091%, corticosteroids 0.161%, hyaluronan 0.159%, and after polysulfated glycosaminoglycan injection 0.394%.²⁸ Although controversial, the authors of the latter study concluded that infectious arthritis following intra-articular injection is rare and the use of prophylactic antimicrobials might be unnecessary.²⁸

2.2.3 Inflammatory response of the synovial structure to bacteria

Factors associated to whether or not a synovial structure can resist an inoculation of microorganisms are organism-related (pathogenicity, virulence and number of organisms);¹¹

immunological status of the animal;⁵ and the presence of foreign material.²⁹ In neonates, a complete or partial failure of passive transfer of immunoglobins is the most common underlying cause that predispose to the development of bacteremia as a complication of umbilical infection, pneumonia or diarrhea.^{14,30,31} The bacteria may settle in joints and their adjacent tissues in foals younger than 6 months of age, most likely because of the low oxygen tension associated with local low blood flow.³¹

The articular inflammatory response to infection is mediated by the innate immune system directed towards the elimination of the microorganism and the repair of damaged tissue.³² Healthy synovial structures are able to control synovial contamination with a certain amount of bacteria and impede their proliferation.⁹ In an in vitro study, 10g of normal equine synovial membrane were capable of neutralizing 100 colony-forming units of *Staphylococcus aureus*.³³ When infection and proliferation of bacteria occur, the joint usually produces a marked inflammatory response, which is determined by factors such as host immunity, number of inoculated bacteria and bacterial virulence.⁹ The degree of tissue damage by the inflammatory response varies according to the host age, host debilitation, duration of infection, bacterial virulence and pre-existent joint disease.⁹

The local inflammatory response is initiated by the release of pro-inflammatory molecules by the injured tissue. These molecules include reactive oxygen species (O₂⁻, OH, NO, and H₂O₂), arachidonic acid metabolites and modified host proteins, which induce the migration and activation of cells such as neutrophils and macrophages, as well as the production of large protective molecules such as complement components and antibodies.³² Different cells including synoviocytes and neutrophils respond to the stimulus by synthesizing and releasing cytokines, which include TNF- α , IL-1, IL-6; as well as free oxygen radicals, and destructive enzymes (lysozyme, elastase, cathepsin G, gelatinase, caseinase, and collagenase).^{34,35} All of these products contribute to the disruption of the blood-synovial barrier. This facilitates the access of many nonspecific mediators, which amplify the inflammatory cascade by further activating the synoviocytes.⁹ Total protein content in the synovial fluid also increases because of the increased capillary permeability, which allows high molecular weight proteins (mainly globulins) to enter the joint.³⁶ Chondrocytes activated by IL-1, TNF- α and free radicals³⁵ release MMPs (stromelysin, collagenase, gelatinase) which decrease proteoglycan synthesis.³⁵ These MMPs are responsible for the cartilage breakdown leading to the development of osteoarthritis.¹⁰

If the amount of inflammatory molecules released by the injured tissue is high, a systemic reaction may be induced, leading to an acute phase response (APR).³² The APR is composed of a series of local and systemic changes that involve many cell types and organs.³⁷ Several proteins called acute phase proteins (APPs) are released into the systemic circulation during an APR.³²

The APPs are produced mainly by the hepatocytes after being stimulated by IL-1, TNF- α , and especially IL-6.³² Their synthesis and release usually begins a few hours after injury.³² The APPs include complement components, clotting molecules, protease inhibitors, and metal-binding proteins.³² The majorly investigated APPs include SAA, C-reactive protein (CRP) and haptoglobin, as well as α 1-acid glycoprotein. In humans, CRP and SAA are the major APPs,³⁸ but in horses CRP has a limited and later response to stimuli and because of this, is not considered a major APP.³⁹ The major APPs have very low or undetectable plasma levels in healthy animals and their concentrations can rise more than 100-1000 times during APR.⁴⁰

Macroscopic changes within the synovial cavity vary depending on chronicity of the infection. The major finding during acute infection is severe synovitis.⁴¹ When osteochondral defects are not present, chronic septic arthritis is characterized by cartilage degeneration. In contrast, if there is a defect in the subchondral bone this may result in infected osteitis/osteomyelitis.⁴¹ During chronic tenosynovitis, when the epitenon is intact, synovial proliferation and adhesion formations are the major findings. On the other hand, if the epitenon is affected, there is usually rapid intra-tendinous collagenolysis.⁴¹ Intra-synovial fibrinocellular conglomerate (pannus) is usually seen in established infections.⁴¹ Pannus commonly develops over areas of villous synovium, and villi may be completely covered.⁴¹ It can then cover avillous synovium and, in advanced cases, articular cartilage or tendon surfaces may be also covered. Pannus may hide foreign material and devitalized tissue; as well as be rich in inflammatory cells, degradative enzymes and radicals.⁴¹ In addition, it can also act as a region for bacterial multiplication and as a barrier for synovial diffusion, limiting synovial nutrition and access for circulating antimicrobial drugs.⁴¹

2.2.4 Diagnosis and monitoring of septic synovitis

The common clinical signs seen in a horse with septic synovitis include heat over the affected structure, synovial effusion and severe lameness.² An increase in body temperature

might be seen in some cases, but is not a common finding.⁴² Horses usually present with a lameness grade of 4 to 5 out of 5.^{20,43,44} There is increased joint effusion with accompanying increased digital pulses in infection of synovial cavities in the distal limb (proximal and distal interphalangeal joints, carpometacarpal/tarsal joints as well as digital flexor tendon sheath and navicular bursa).⁴³ The administration of intra-synovial local anesthetic does not always alleviates lameness.⁴³

Radiographic assessment of septic synovial structures is recommended.^{20,43} It may reveal increased width of the joint space, soft tissue swelling, subchondral bone lysis and osteophytosis.^{20,43} In some cases radiographic changes are evident early in the course of sepsis; however, there is frequently a time lag from injury to evidence of radiographic changes.⁴² Consequently, if there is no radiographic evidence of osteomyelitis before initiating treatment, it is recommended that radiographs be repeated in refractory cases.³¹

Bacteria are not always cultured from septic synovial samples^{11,24} and this is not necessarily associated with parenteral administration of antimicrobials before synoviocentesis.^{2,45} Sequestration of the bacteria into the synovial membrane³ and the bactericidal effect of synovial fluid^{46,47} may be reasons for obtaining a negative culture. Other factors that may contribute to a negative culture are sample handling (samples should be processed within 1 h after collection⁴⁸), low number of organisms present and low virulence of organisms.^{31,48} Reported success rates in obtaining a positive culture from septic synovial fluid range from 22% to 79%.^{2,11,24,43,48,49} Synovial fluid samples are recommended to be placed into a bacterial culture bottle to maximize the likelihood in obtaining a positive result.³¹ The use of blood culture bottles⁵⁰ and enrichment broth prior to culture² have been reported to be advantageous⁴⁸. Other studies have reported that culture enhancement techniques with blood culture media allow for bacterial isolation in 87% to 100% of septic joints.^{51,52}

In conjunction with clinical signs and bacterial culture, diagnosis of synovial sepsis is facilitated by the use of synovial fluid analysis.² Total protein concentration and total nucleated cell count are measured in synovial fluid.⁵³ Cytology of the synovial fluid precipitate is commonly advantageous for obtaining an accurate diagnosis.⁵³ The use of Gram stain of synovial fluid can sometimes expose bacteria and confirm sepsis.⁵³ In addition, the percentage of neutrophils obtained during cytology can help in diagnosis synovial sepsis.⁵³ Degenerative neutrophils are not commonly encountered, but when found are highly suspicious of sepsis.^{11,24,53}

The reference values for normal synovial fluid have been documented as total protein <25 g/L, nucleated cell count <1 x 10⁹ cells/L and percentage of neutrophils being <10%.^{54,55} Sepsis has typically been considered when synovial fluid yields a total protein ≥40 g/L, nucleated cell count ≥30 x 10⁹ cells/L and percentage of neutrophils ≥80%.^{53,54} However, other authors have suggested synovial sepsis with values of nucleated cell counts ≥ 5 x 10⁹ cells/L^{4,8,11,19} and total protein >30 g/L.² The latter ranges can be found during non-septic synovitis and should be used with caution and together with clinical signs of septic synovitis.⁵³

Few studies have focused on assessing and monitoring treatment for septic synovitis. A subjective grading system has been developed in human medicine to predict the efficacy of treatment based on radiographic findings and findings during exploratory arthroscopy.⁵⁶ In a retrospective study investigating prognostic indicators for adult horses with synovial sepsis, synovial fluid samples obtained 4 to 6 days after initiating surgical treatment for septic arthritis were classified as ‘unremarkable’ (NCC ≤1 x 10⁹ cells/L, and neutrophils ≤30%), ‘synovitis’ (NCC >1 to <5 x 10⁹ cells/L, or neutrophils >30% to <80%), and ‘sepsis’ (NCC ≥5 x 10⁹ cells/L, or neutrophils ≥80%).¹² It was found that horses classified at 4 to 6 days postsurgical as ‘synovitis’ and ‘sepsis’ had a reduced percentage of survival to discharge (84%) and return to athletic function (47%) than horses classified as ‘unremarkable’ (100% and 90%, respectively). In the same study it was suggested that concerns regarding iatrogenic exacerbation of inflammation by surgical manipulation might be unfounded as 39% of the horses in the study returned to normal values rapidly after surgery and were classified as ‘unremarkable’ at 4 to 6 days. In another retrospective study that investigated factors associated with survival to hospital discharge after endoscopic treatment for synovial sepsis in horses, a total protein concentration of 50-55 g/L obtained after arthroscopic lavage was associated with reduced likelihood of survival to hospital discharge.¹⁹ Also, persistent synovial infection during treatment demonstrated by a positive bacterial culture have shown to be associated with a reduced prognosis for survival.²

2.3 Treatment options for septic synovitis and their effects on common synovial inflammatory markers

When treating synovial infection removal of debris, foreign material, devitalized and contaminated tissue, as well as destructive enzymes and radicals should be achieved by some

form of drainage or lavage.^{41,57,58} Closed suction drainage is advantageous as it is thought to remove damaging waste products continuously and maintains patient comfort by avoiding increased joint effusion.²³ A method suggested for early cases of septic synovitis is ‘distention-irrigation’ in which a large-gauge needle is inserted into the synovial cavity and infusion and aspiration of an irrigating fluid is done.⁵⁸ As an alternative to the latter method, through-and-through joint lavage is used and allows for a continuous flow of an irrigation fluid.⁵⁸ On well established infections fibrin clots can be too large to be flushed by through-and-through joint lavage⁵⁷ and arthrotomy or arthroscopy are necessary to treat these cases successfully.⁵⁸ Arthrotomy allows extensive lavage and drainage by leaving the surgical incisions open.^{59,60} However, arthrotomy complications include premature healing of the arthrotomy site or, in contrast delayed healing.⁶¹ In acute infections, arthroscopy, synovectomy, and lavage are preferred over arthrotomy and lavage.^{23,61} Arthrotomy and lavage eliminated infection more effectively and provided better drainage than arthroscopy, synovectomy and lavage during experimentally induced *Staphylococcus aureus* infection to the tarsocrural joint.⁶¹ However, joints that were treated by arthrotomy had a greater rate of ascending bacterial contamination.⁶¹ Therefore, arthroscopy with synovectomy and lavage is preferred in cases of septic arthritis.^{15,23,61}

Variations of these techniques have also been described and include arthrotomy followed by insertion of closed suction³ or open passive drains,⁶² open drainage^{59,61,63} and arthroscopy followed by closed suction drainage,^{8,23} fenestrated drains,¹⁷ multi-fenestrated indwelling lavage systems,⁶⁴ or creation of an open draining wound.⁹ In chronically infected joints, and where amenable, ankylosis can be performed if the joint function is irreversibly compromised.^{43,65} In chronic cases of sepsis of the common digital extensor sheath, complete surgical resection of the affected tendon and ablation of the tendon sheath has been successfully performed obtaining good prognosis for return to soundness.⁶⁶

Although its use is not common and still controversial, dimethylsulfoxide (DMSO) has been used to treat septic arthritis successfully.⁴³ It has been proposed that DMSO binds and neutralizes oxygen-derived free radicals and suppresses prostaglandin production and therefore reduces further cartilage degradation and decreases inflammation.^{43,67}

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to alleviate lameness by controlling pain and inflammation⁶⁸ and as an analgesic administered peri-

operatively.⁶⁹ Phenylbutazone and flunixin-meglumine are commonly used.⁶ These are non-selective cyclooxygenase (COX) inhibitors and can cause renal and gastrointestinal toxicity.^{70,71} The risk of toxicity associated with non-selective COX inhibitors can increase when dehydration is present, therefore this should be monitored when phenylbutazone and flunixin-meglumine are administered.^{6,71} Recently firocoxib (an specific inhibitor of COX-2 isoenzyme⁷²) has been used when long-term administration of NSAIDs is needed.^{73,74} The use of firocoxib was associated with low risk of developing NSAIDs toxicity in horses⁷³ and proved to have similar effects in reduction of lameness as phenylbutazone.⁷⁴ Intra-articular corticosteroids⁶⁰ may be used after sepsis has resolved, as well as hyaluronic acid^{75,76} and polysulphated glycosaminoglycans⁷⁶ to restore the joint environment and prevent or treat further degenerative joint disease.

Strict stall rest has been advised during the acute phases of inflammation and heavy bandages can be placed if necessary.^{58,60} Bandages can serve to reduce and control swelling by providing compression.⁶ If wound care and local therapy are needed daily, bandages are usually changed on a daily basis to allow treatment until synovial infection resolves and wounds are healed.⁶ It has also been recommended that after resolution of infection, the patient should be confined for several weeks and reintroduced to exercise slowly.⁵⁸ Controlled exercise with the addition of physical therapy that may include passive range-of-motion exercises, can be performed once infection and synovitis have resolved.⁶

2.3.1 Arthroscopic lavage

Arthroscopy is the preferred procedure to lavage joints when sepsis is present. It is a minimally invasive procedure which allows good visualization of the joint structures, access to remote parts of the joint, as well as removal of fibrin, debridement of infected bone and synovectomy.^{23,41,61} The infected synovial cavity can be assessed for concurrent damage to cartilaginous, osseous and soft tissue structures to determine prognosis.^{4,18,56} Arthroscopy also allows lavage with high volumes facilitating removal of inflammatory mediators from the infected synovial cavity.⁷⁷

The inflammatory response of gas or fluid distention during arthroscopy in healthy equine joints has been reported.⁷⁸ Tarsocrural joints were distended with warm carbon dioxide (CO₂) gas or lactated Ringer's solution (LRS) both at set pressures of 24 mmHg for 30

minutes.⁷⁸ Synovial fluid samples were then collected at 0, 6, 12, 24 and 48 h after the procedure. At 6 h, all samples from both gas and LRS distention were cloudy and red in color. Samples obtained from gas distended joints acquired an orange color which improved over time in contrast to LRS distended joints that remained red in color until the end of the study (48 h). Values of nucleated cell counts were similar between groups reaching values as high as 20 to 25 x 10⁹ cells/L at 6 and 12 h after arthroscopy and then slowly decreasing in values. However, they were still significantly different to baseline values (time 0 h) at 48 h. The percentage of neutrophils were >80% at 6 and 12 h and slowly decreased to values ~60% by 48 h. The total protein concentration was also similar in both groups and reached its peak at 24 h after the procedures (specific values not reported). The only significant differences reported between groups were the levels of hemoglobin and leukocyte oxidative burst, which were lower in the gas distended group than in the LRS distended group. This indicates that CO₂ arthroscopy produced less intra-articular bleeding and activation of infiltrating leukocytes than LRS distention.

Another study evaluated the changes in synovial fluid after arthroscopic partial synovectomy in horses.⁷⁹ Lavage of the middle carpal joints was performed with 7.2 L of LRS at a rate of 110 mL/minute and synovectomy was performed on one limb and the contralateral received only arthroscopic lavage with the same amount of LRS. Synovial fluid samples were collected before arthroscopy and 14, 21 and 28 days after arthroscopy. Because sampling was not done immediately after the procedure, the acute inflammatory response of the joint to arthroscopy lavage and arthroscopic partial synovectomy was missed in this study. Samples in both groups were turbid or slightly hemorrhagic for 4 weeks and samples at 21 and 28 days were clearer than those obtained at 8 and 14 days. At 8 days after arthroscopic lavage and after arthroscopic lavage and synovectomy, total protein (27.6 ± 6.5 g/L and 29.8 ± 4.1 g/L respectively), nucleated cell counts (0.81 ± 0.57 x 10⁹ cells/L and 1.21 ± 1.07 x 10⁹ cells/L respectively) and percentage of neutrophils (33 ± 25%) were significantly increased from preoperative values. By the end of the study (28 days), synovial fluid parameters were still significantly higher than baseline values in some horses.

2.3.2 Through-and-through joint lavage

Lavage of a synovial structure by placing at least two needles and performing a through-and-through joint lavage has been shown to be effective in treating septic arthritis.^{43,57,60} In chronic cases, where pannus and fibrin are present, it has been recommended to place several needles in different synovial pouches to obtain a more thorough lavage of the entire synovial cavity.⁴³ The mean number of through-and-through joint lavages required to resolve synovial sepsis in a clinical study was 3 (range 2-15).⁵⁷ However, in this study, clinical cases were only treated with joint lavages and systemic antimicrobials; antimicrobials were not administered regionally.

The effect of through-and-through joint lavage with four antiseptic solutions was evaluated in healthy equine tarsocrural joints.⁸⁰ Three concentrations of povidone-iodine (0.1%, 0.2%, and 0.5%) and one concentration of chlorhexidine (0.5%) were compared. Both 0.5% povidone-iodine and 0.5% chlorhexidine induced severe lameness, soreness at palpation and limb enlargement. Results from this study concluded that 0.1% povidone iodine in balanced electrolyte solution is the only bactericidal joint lavage solution recommended in the horse. The other solutions produced synovitis of different degrees and particularly 0.5% chlorhexidine produced a significant increase in nucleated cell counts, percentage of neutrophils and cartilage damage visible on histopathology. Balanced electrolyte solution was used as a control solution and induced synovitis at 24 h after lavage with nucleated cell counts reaching up to 38×10^9 cells/L, total protein reaching up to 5.3 g/L and approximately 80% of neutrophils (non-degenerative). In another experimental study, 0.1% povidone-iodine was used in joints experimentally infected with *Staphylococcus aureus* to evaluate its efficacy as bactericidal compared to balanced electrolyte solution.⁸¹ It was concluded from this study that through-and-through joint lavage with 0.1% povidine-iodine had no advantage over lavage with balanced electrolyte solution for the treatment of infectious arthritis in the horse.

Chlorhedixine at a concentration of 0.05% was evaluated as a single lavage solution in healthy tarsocrural joints resulting in synovial ulceration, inflammation and abundant fibrin accumulation.⁸² Therefore, it was not recommended for use in equine joints. In the same study, through-and-through joint lavage with LRS was used as control. Total protein in control joints was not affected by LRS lavage, but nucleated cell counts and percentage of neutrophils were

significantly increased at 24 h after lavage with LRS (up to 8.3×10^9 cells/L and up to 71%; respectively). Later on, the effect of through-and-through joint lavage with potentiated chlorhexidine solution (0.0005% chlorhexidine in EDTA-Tris) was evaluated in healthy tarsocrural joints of horses.⁸³ It was found that potentiated chlorhexidine had no detrimental effects to synovium or articular cartilage but produced synovitis that resolved at 8 days post-lavage. In the same study, phosphate-buffered saline solution was used as control lavage solution and had similar effects as potentiated chlorhexidine producing a synovitis characterized by increased nucleated cell count (25.7×10^9 cells/L \pm 28), total protein (3.0 g/L \pm 0.6) and percentage of neutrophils (68.7% \pm not reported).

2.3.3 Antimicrobial administration

Systemic antimicrobials have been recommended when treating synovial sepsis for an extended period of at least 3 weeks.^{17,42} When synovial sepsis is suspected, broad-spectrum antibiotics are given systemically until results of bacterial culture and bacterial antimicrobial sensitivity are available.⁶ The antimicrobials given initially are chosen based on the suspected cause for the synovial infection (e.g. iatrogenic infection or by a wound).⁶ To obtain a good broad-spectrum coverage, antimicrobials with Gram-positive (e.g. β -lactam or a cephalosporin) and Gram-negative (e.g. aminoglycosides or fluorquinolones) spectrum should be combined.⁶ When iatrogenic infection is suspected, aminoglycosides are commonly chosen and particularly amikacin has shown to be highly effective against 95% of bacteria isolated from horses with postoperative septic arthritis including *Staphylococcus* spp., Enterobacteriaceae and *Pseudomonas* spp.⁸⁴ If infection with an anaerobic bacteria is suspected (e.g. *Bacteroides*) the antimicrobial selection should include metronidazole.⁶ If a positive bacterial culture is available, systemic antimicrobial therapy should be based on bacterial sensitivity results.⁶

Bacterial resistance to antimicrobials is a challenging clinical scenario in human and veterinary medicine.^{6,85} Methicillin-resistant *Staphylococcus aureus* (MRSA) is usually a nosocomial infection and can be zoonanthroponotic (reverse zoonotic disease).⁸⁵ Unfortunately in equine medicine a variety of antimicrobial drugs against MRSA are not available.⁶ In horses, imipenem and vancomycin have been used successfully to treat orthopedic MRSA infections.⁸⁶ In humans, resistant infections can be treated with different antimicrobials like oxazolidinone,

linezolid, pristinamycin, ortavancin, dalbavancin, romaplanin, tigecycline, BAY73-7388 and a newer antimicrobial named daptomycin.⁶

Systemic antimicrobials are commonly combined with local administration of antimicrobials either by intra-synovial injections⁵⁹ or by regional limb perfusion when treating synovial sepsis.⁸⁷ Intra-synovial administration of antimicrobials achieves an immediate synovial concentration of the antimicrobial above the MIC for common bacterial equine isolates.⁸⁸⁻⁹⁰ Low quantities of antimicrobial are needed to obtain high concentrations.⁸⁸⁻⁹⁰ However, intra-articular injections of antimicrobials have shown to produce a mild transient inflammatory reaction that usually resolves without further treatment.⁸⁸⁻⁹⁰

Various antimicrobials have been shown to be safe for intra-synovial administration.^{3,84,88,89} Ceftiofur can be safely administered intra-articularly in horses and it is commonly used based on bacterial sensitivity.⁸⁸ Aminoglycosides have become the preferred intra-articular antimicrobial drugs for the prevention and treatment of equine joint sepsis.⁸⁴ Intra-articular administration of gentamicin achieves concentrations in synovial fluid above the MIC for many common equine bacterial isolates for more than 24 h.⁸⁹ Gentamicin administered intra-articular at a dose of 150mg results in a higher concentration (about 1000-fold) than when given systemically.⁸⁹ Gram-negative bacteria can be resistant to gentamicin and this has reduced its use, however it is still effective against *Staphylococcus* spp. and *Streptococcus* spp.⁹¹ In the other hand, amikacin has poor activity against *Streptococcus* spp. but is more effective than gentamicin against *Staphylococcus* spp. and Gram-negative bacteria.⁹¹ Intra-articular administration of 250 to 500 mg of amikacin sulphate every 24 or 48 h is commonly used for the treatment of septic arthritis.³ A single administration of 500 mg of amikacin into the radiocarpal joint of horses produced synovial concentrations of amikacin that remained above the reported MIC for most equine pathogens for 72 and 48 h in normal and inflamed joints, respectively.^{90,92} Amikacin produced toxic effects on equine chondrocytes in explant cultures.⁹³

Repeated intra-synovial administration of antimicrobials is most commonly performed via arthrocentesis every 24 to 48 h when treating septic arthritis in horses.³ Repeated intra-articular administration of amikacin significantly increased total protein (43.2 ± 9.5) and nucleated cell counts ($2.49 \pm 2.48 \times 10^9$ cells/L), and total protein values were higher than the reference values for septic arthritis in some horses.⁹⁴

Antimicrobial drugs can also be safely delivered by intra-synovial constant rate infusions systems.⁹⁵⁻⁹⁷ The advantage of using intra-synovial constant rate systems for infusion of antimicrobials when treating septic synovitis include avoiding daily synoviocentesis⁶ as well as lavage of the joint by the same system.^{87,97,98} Gentamicin has proved to be safe to deliver by intra-synovial constant rate infusions obtaining high concentrations above the MIC for most equine bacterial isolates⁹⁶ not only in synovial fluid, but also in associated synovial structures (subchondral bone synovial membrane, and joint capsule).⁹⁵ Other antimicrobials that have been used by an intra-synovial constant rate system are amikacin and ticarcillin with clavulanate obtaining up to 92% of horses that recovered successfully from septic synovitis.⁹⁷

Intra-articular antimicrobial-impregnated beads are used in chronic cases of synovial sepsis where osteomyelitis is present.^{99,100} In one report, where all cases were refractory to standard treatments, antimicrobial-impregnated beads were used and left for 14 days intra-synovially.¹⁰⁰ In this report, 11 out of 12 horses recovered successfully and returned to previous athletic function.

Regional limb perfusion with antimicrobial drugs has been shown to achieve high and prolonged levels of the drug in synovial fluid¹⁰¹ and to eliminate infection successfully.^{87,102} The use of regional limb perfusion has lead to similar rates of survival to discharge and return to previous performance than with other techniques used for regional delivery of antimicrobials.⁸⁷ Regional limb perfusion can be performed either by intra-osseous regional limb perfusion (IORLP)¹⁰¹ or by intra-venous regional limb perfusion (IVRLP).¹⁰³ Both techniques are widely used, however IVRLP can have fewer complications than IORLP⁸⁷ and therefore is preferred.⁸⁷ With IVRLP, high concentrations of antibiotics are obtained in bone tissue and this might increase the success in treating synovial sepsis with concurrent osteomyelitis.⁸⁷

2.4 Biology of serum amyloid A

Serum amyloid A is a characteristic and sensitive acute phase protein in several animal species and humans.¹⁰⁴ Serum amyloid A is an apolipoprotein and the majority of SAA circulates in plasma bound to the fraction 3 of high-density lipoprotein (HDL3) and a minority circulates in a free form.^{105,106} It is primarily synthesized by the liver, but it is also expressed in several other tissues which will be discussed in the following sections of this literature review.¹⁰⁷ During acute

inflammation approximately 2% of the total hepatic protein synthetic capacity is dedicated to produce SAA.¹⁰⁸ Serum amyloid A induction is mediated by IL-1, IL-6 and TNF α ; as well as other cytokines produced by activated macrophages.¹⁰⁹ It undergoes hepatic degradation¹¹⁰ and has a short plasma half-life of 75 to 80 minutes.¹¹¹ Serum amyloid A has been associated with amyloidosis in humans and horses and this disease has been classified according to the protein that makes the majority of the deposits (for deposits of SAA is AA amyloidosis); and according to whether the disease is primary (originates from a disorder in the immune cell function) or secondary (originates during chronic inflammatory diseases).¹¹² During chronic inflammatory disease such as rheumatoid arthritis in humans, SAA is persistently increased and has an important role in the development of AA amyloidosis, which is a type of amyloidosis described as an abnormal deposition of SAA in the extracellular space of several organs.¹¹³ Serum amyloid A is the precursor for amyloid protein AA that is the main fibril protein in secondary (reactive) amyloidosis in humans.¹¹³ In horses, reactive amyloidosis has been induced after repeated stimulation by microbial agents for the production of antibodies and the complete amino acid sequence of protein AA has been reported by this procedure.¹¹⁴ Also, in humans, SAA has been well correlated with the risk of developing cardiovascular disease and this suggests SAA has a role in the pathogenesis of arteriosclerosis.^{115,116} The functions of SAA are poorly understood and it has been suggested that SAA may serve as a local defense mechanism against inflammatory stimuli during the time lapse between the stimuli and the mounting of a systemic (hepatic) inflammatory response.¹¹⁷

2.4.1 Molecular architecture, genes expression and isoforms

Equine SAA is a 9-11 kilodalton, hydrophobic,¹¹⁸ apolipoprotein.^{105,119} The primary structure of SAA consists of 110 amino acid residues with microheterogeneities found on positions 16, 44, and 59.¹¹⁴ This demonstrates the existence of more than one SAA gene in the horse.¹¹⁸ There is a marked homology within human SAA and SAA in other several animal species, showing that SAA is a well-conserved protein within species.^{118,120} The conservation of SAA through evolution is demonstrated with equine SAA which has a high homology with SAA from dog (80.6%), mink (77.5%), human (76.9%) and duck (71.9%).¹²¹ This homology of SAA proteins and genes within eutherian mammals¹²² includes also other vertebrates like

marsupials¹²³ and fish,¹²⁴ as well as invertebrates as the echinoderm *Holothuria glaberrima* (Sea Cucumber); suggesting that SAA has survived for more than 500 million years.¹²⁵ The SAA complementary deoxyribonucleic acid (cDNA) in the horse is 480 nucleotides in length encoding an SAA precursor protein of 128 amino acids.¹²¹ To yield a mature SAA protein of 110 residues, post-transcriptional cleavage of the precursor protein occurs after glycine at position 18.¹²¹

Extra-hepatic synthesis of SAA has been reported in various species such as rabbits, minks, mice,¹²⁶ bovine,¹⁰⁷ horses¹²⁷ and humans.^{126,128} Different SAA isoforms have been documented for these species. Isoforms SAA1 and SAA2 are synthesized by the liver, while SAA3 is mainly expressed at extrahepatic sites.¹¹⁷ The ‘acute-phase’ SAA includes the isoforms SAA1 and SAA2, which majorly circulates bound to HDL3 and a smaller quantity circulates free during inflammation.¹²⁹ Expression of SAA3 (a pseudogene in humans)¹³⁰ has been demonstrated in adipose tissue¹³¹ and in the mammary gland of cattle,¹³² as well as the isoform SAA3 has been quantified in colostrum from bovine, equine and ovine¹³² as well as in bovine milk during mastitis.¹³³ In horses, SAA3 has been demonstrated in peritoneal fluid,¹³⁴ colostrum¹³² and synovial fluid.¹²⁷ Also, SAA3 messenger ribonucleic acid (mRNA) expression has been demonstrated on equine endometrium during uterine *Escherichia coli* infection.¹³⁵

A ‘constitutive’ SAA is a fourth isoform (SAA4) found on several tissues within the body, is minimally induced during inflammation and has been found bound to HDL in healthy individuals.^{107,117} In healthy horses, moderate (>1% of the hepatic expression) SAA4 mRNA expression was found in the mammary gland, lung, synovial membrane, pancreas, thymus, thyroid and uterus.¹⁰⁷ Low expression (<1% of the hepatic expression) was also documented on adipose tissue, adrenal gland, aorta, brain, colon, jejunum, stomach, heart, kidney lymph nodes, ovary, testis, prostate, skeletal muscle, cardiac muscle, skin and spleen.¹⁰⁷ Constitutive SAA (SAA4) has only a degree of 50% of similarity in the amino acid sequence with the acute-phase SAA (SAA1 and SAA2) suggesting that constitutive SAA may represent a separate protein family having different functions.¹¹⁷ The differences in amino acid sequences of SAA proteins within different tissues have led to suggest that each SAA protein may adapt to a particular function in each particular tissue.¹²⁸

2.4.2 Transport through the bloodstream and metabolism of serum amyloid A

Serum amyloid A is an apolipoprotein that circulates in plasma in its majority bound to HDL3.^{105,106} In individuals with normal levels of SAA, about 0.1% of the total HDL3 is bound to SAA.¹⁰⁶ Just after SAA is synthesized, it is primarily incorporated into HDL3 (occupying about 1% of the total HDL3 protein in serum during inflammation), and a minority can be associated with other lipoproteins containing apoN-100, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). During inflammation, SAA increases to high concentrations in serum and becomes the major apolipoprotein bound to HDL3, but some SAA also remains unbound in serum.¹³⁶ During an APR, the concentration of free SAA (not bound to HDL3) increases in serum and can form aggregates.¹³⁷ It has been suggested that the free form of SAA is the form that binds to a variety of receptors, which will be described in the following sections.¹³⁷ Serum amyloid A is degraded in the liver¹¹⁰ after a short plasma half-life. This has been estimated to be from 75 to 80 minutes in mice, with a 95% clearance from plasma 6 h after the synthesis has stopped.¹¹¹ The exact half-life of SAA in horses is unknown but its plasma levels increase 3 to 6 h after an inflammatory stimulus, peaking on day 3 and returning to baseline levels by day 4,^{119,138} demonstrating that equine SAA may have a similar short half-life as the one described in mice.⁴⁰

2.5 Serum amyloid A biological functions

Most of the research on the biological functions of SAA originates from the human literature. The reported functions of SAA in mammals are varied and poorly understood.¹⁰⁷ In addition, many pitfalls have been identified in these studies.¹³⁹ Serum amyloid A may have different functions and could serve as a first defense mechanism against inflammatory stimuli.¹¹⁷ It can bind to several cell surface receptors and activate the target cells, but no SAA-specific receptor has been identified.¹³⁹ It has been suggested that SAA may be important to modulate several immune-inflammatory reactions that include inhibition of the activation of polymorphonuclear leukocytes,¹⁴⁰ induction of cyclooxygenase metabolites in mononuclear leukocytes,¹⁴¹ and induction of mononuclear and polymorphonuclear leukocyte phagocytosis and chemotaxis.^{142,143} Protective functions of SAA have also been documented. Serum amyloid 3 can

induce intestinal mucin production in human intestines causing a reduction in the adherence of enteropathogenic *Escherichia coli* to the HT29 human intestinal cells.¹⁴⁴ Also, SAA3 has cytokine-like properties being chemotactic to various cell types and binding to the surface of Gram-negative bacteria.¹⁴⁵ Furthermore, SAA from porcine milk have shown to have LPS-binding capacity.¹⁴⁶ It has also been reported that SAA has several functions associated with the maintenance of normal functions through its effects on lipid transportation,¹²⁸ and collagenase synthesis.¹⁴⁷ The specific known biological functions of SAA will be discussed in the following sub-sections.

2.5.1 Regulatory mechanisms of serum amyloid A

Interleukin 1 β is a key cytokine during inflammation and two separate signals are necessary to induce its secretion.¹⁴⁸ The first signal is necessary to induce the synthesis of pro-IL-1 β and the second signal induces the formation of an intracellular protein platform called the inflammasome. The inflammasomes mediate the conversion of pro-IL-1 β into its biologically active form.^{148,149} This conversion is mediated by activation of caspase-1 protease.^{148,149} Serum amyloid A induces the release of mature IL-1 β by stimulating the two necessary signals in neutrophils,¹⁵⁰ mast cells,¹⁵¹ macrophages¹⁵² and dendritic cells.¹⁵³ The regulation of SAA over production of mature IL-1 β has been shown to be predominantly by activation of the inflammasome NLRP3.¹⁵²

2.5.2 Serum amyloid A role in immunity

Serum amyloid A is a mediator of the innate immune system and also regulates the adaptive immune response.^{32,142} Its specific functions depend on the type of target cell and the local concentration of SAA.^{32,142} In humans, neutrophils are induced to produce TNF α , IL-1 β , and IL-8 by SAA;¹⁵⁰ while mast cells are induced to produce TNF α and IL-1 β .¹⁵¹ SAA-induced mononuclear cells express several pro-inflammatory cytokines (TNF α , IL-1 β , IL-6, and IL-8) as well as the growth-promoting granulocyte-macrophage colony-stimulating factor (GM-CSF).¹⁵⁴ Serum amyloid A also serves as a chemo-attractant for polymorphonuclear leukocytes and monocytes,¹⁴² and induces directional migration of human mast cells.¹⁵⁵

Serum amyloid A is a powerful pro-inflammatory mediator as it can bind and activate several structurally diverse receptors as well as induce activation of several signaling pathways.^{156,157} The activation of Toll-like receptor 4 (TLR4) by SAA increases the production of nitric oxide by macrophages.¹⁵⁸ By activation of formyl peptide receptor-like 1 (FPRL1) in neutrophils, SAA induces release of FPRL1-dependent calcium, IL-8 and TNF α .¹⁵⁹ In monocytes, the activation of the same receptor by SAA induces secretion of both anti- and pro-inflammatory cytokines (IL-10 and TNF α , respectively).¹⁶⁰ By FPRL1, SAA also induces phagocyte migration to the injured tissue.¹⁶¹

2.5.3 Anti-inflammatory properties of serum amyloid A

The known anti-inflammatory properties of SAA are promoting the release of interleukin 10 (IL-10)¹⁵⁴ and inhibiting the oxidative burst in neutrophils.¹⁴⁰ Serum amyloid A has also an effect on the proliferation of regulatory T cells.¹⁶² This effect has been suggested to be indirect by inducing a microenvironment that supports proliferation of regulatory T cells.¹⁶²

2.6 Serum amyloid A as a marker of systemic inflammation in horses

Serum amyloid A is a good marker of systemic inflammation. It has very low basal values that can increase up to 1000 times during an APR.¹¹⁹ Its serum levels are not affected by sex (except for a moderate increase after parturition), and mild changes have been reported between different age groups.¹¹⁹ Concentrations of SAA can rise to different ranges depending on the amount of tissue damage. These concentrations decrease quickly after resolution of the tissue damage because of the SAA's short half-life.^{40,119} In humans, its concentration in serum rises to higher levels in response to bacterial stimulus than to viral infection or non-infectious inflammation.¹⁴¹

The basal values of SAA in serum of healthy horses are <30 mg/L,^{119,163-165} with certain variability observed depending on the assay used.⁴⁰ In horses with experimentally-induced systemic inflammation (by intramuscular administration of turpentine oil) SAA concentrations rose within 6 h and reached their serum peak concentration 2 days later with a 20-fold increase from pre-treatment values.¹¹⁹

A recent study evaluated the clinical application of the assessment of SAA in a specialized equine practice.¹⁶⁶ A total of 212 horses were included and classified as clinically normal or clinically abnormal. The clinically abnormal horses had a variety of infectious or inflammatory conditions and all of them had significantly higher concentration of SAA in serum compared to the clinically normal horses. The authors of the study concluded that the assessment of SAA concentrations in clinically abnormal horses might be of value for monitoring disease and as a prognostic indicator.¹⁶⁶

Serum amyloid A, fibrinogen, haptoglobin and alpha₁-acid-glycoprotein were recently detected in high concentrations in saliva of horses with systemic inflammation, suggesting that measurement of these APPs in saliva might be useful in monitoring disease.¹⁶⁷ In another study on mares on late pregnancy where placentitis was induced; SAA and haptoglobin concentration in serum increased rapidly and remained increased until abortion occurred. This finding suggested that these APPs might be useful in monitoring placentitis.¹⁶⁸ Moreover, SAA and surfactant protein D were increased in experimental bacterial pneumonia in horses, implying they might be useful in monitoring the clinical progression of this disease.¹⁶⁹

In a report on horses with colic, the quantification of SAA systemic concentrations helped in identifying horses with colic attributed to disease that have inflammation as a primary component of pathogenesis, as enteritis, colitis, peritonitis, or abdominal abscesses.¹⁷⁰ Furthermore, SAA were significantly increased in peritoneal fluid and serum from horses with colic. In the latter study, there was a strong correlation between paired serum and peritoneal fluid SAA concentrations.¹³⁴ The authors concluded that SAA in peritoneal fluid and serum are potential valuable diagnostic markers for inflammatory abdominal conditions.¹³⁴

Contrary to these results, the use of SAA in serum from foals with *Rhodococcus equi* pneumonia was not reliable as an ancillary diagnostic tool or as a screening test for early detection of disease during the first month post-partum.¹⁷¹ Similarly, SAA was found to remain low during experimental infection of horses with *Strongylus vulgaris* larvae.¹⁷²

2.7 Serum amyloid A in synovial fluid

The role of serum amyloid A in synovial fluid is still not well understood.¹⁷³ Serum amyloid A is known to be involved in several pro-inflammatory reactions that may lead to

destruction of the tissues within the joint.^{173,174} These are mainly induction of chemokines, attraction of leukocytes, angiogenesis, and induction of MMPs synthesis.^{173,174} Furthermore, SAA is involved in cholesterol metabolism in inflamed tissues and therefore, in the local release of pro-inflammatory molecules.¹³⁹

2.7.1 The role of serum amyloid A in rheumatoid arthritis

Serum amyloid A has been detected in the synovial membrane from rheumatoid patients as well as in their synoviocytes.¹⁷⁵ The presence of SAA in human synovial fluid has an important role in the pathogenesis of inflammatory arthritis (rheumatoid arthritis, psoriatic arthritis, sarcoid arthritis, and undifferentiated arthritis) by inducing the production of MMPs, which are associated with cartilage degeneration.^{174,176,177} It induces the synthesis of IL-6,¹⁷⁸ IL-23 p19 and p40¹⁷⁹ in synovial fibroblasts from patients with rheumatoid arthritis. The Th17 type of immune response is important during rheumatoid arthritis and SAA increases expression of IL-1 β by the inflammasome NLRP3,¹⁷⁹ which promotes the development of Th17 response.¹⁵³ Also, in synovial fibroblasts, SAA induces IL-6 and IL-8 expression by activation of the receptor for the advanced glycation end products (RAGE) and acting as a pro-inflammatory cytokine.¹⁸⁰

2.7.2 The role of serum amyloid A in osteoarthritis

Osteoarthritis is considered a progressive degenerative disease with constant cartilage breakdown, osteophyte formation and subchondral bone thickening.¹⁸¹ Recently, SAA functions during both inflammation and metabolic pathways have been investigated.¹⁸² Serum amyloid A was detected in sections from osteoarthritic cartilage¹⁷⁴ as well as its concentrations were increased in plasma from osteoarthritic patients.¹⁸³ Also, SAA in synovial fluid and plasma increases with radiographic progression of osteoarthritis in human patients.^{183,184} The level of SAA in plasma was higher than in synovial fluid in a range of 3 to 44 fold indicating a passive diffusion of SAA from circulating fluid to the synovial fluid and not the opposite.¹⁸³

The induction of cytokines (IL-6 and IL-8) and MMPs (MMP-1, MMP-3 and MMP-13) by SAA has been described during osteoarthritis¹⁸³ similar to what has been described during rheumatoid arthritis.¹⁷³ The induction of different inflammatory mediators by SAA supports an

important role of SAA in the pathophysiological process of osteoarthritis in humans.¹⁸³ Although only slight increases in serum and synovial fluid SAA concentrations have been reported in humans with osteoarthritis^{185,186} it has been suggested that SAA could be an earlier marker of joint damage than radiographic imaging as well as a prognostic and disease activity indicator.^{187,188} In horses, only one study has reported SAA during osteoarthritis as well as osteochondrosis¹⁸⁹ indicating low or undetectable concentrations in serum and synovial fluid. Interestingly, in the latter study more synovial fluid samples from osteoarthritic joints had SAA concentrations above the detection limit of the assay than serum samples, which may reflect local synthesis of SAA in absence of a systemic response.¹⁸⁹

Contrary to the known anti-inflammatory effects of glucocorticoids, these medications can induce SAA secretion (a pro-inflammatory mediator) in osteoarthritic joints.¹⁸³ This effect of glucocorticoids is not yet well understood.¹⁸³

2.7.3 Serum amyloid A in synovial fluid from horses

Three SAA isoforms have been recognized in the equine serum.^{127,129} Five isoforms were reported in synovial fluid in experimental arthritis induced by intra-articular injection of lipopolysaccharides (LPS).¹²⁷ Three of these isoforms were also found in serum and the remaining two only in synovial fluid. These findings indicate that isoforms produced by the liver (SAA1 and SAA2) can access the synovial cavity and that synthesis of equine SAA also occurs intra-articularly (SAA3) similar to what has been demonstrated in articular chondrocytes and synoviocytes in rabbits¹⁴⁷ and humans.^{175,176,183}

Serum amyloid A concentration in synovial fluid in horses may serve as a good marker for septic synovitis as it has been detected in high concentrations in synovial fluid during septic arthritis and after experimentally induced synovitis by LPS (experimental model for septic arthritis/synovitis).^{127,189} In healthy equine joints, synovial concentrations of SAA are frequently undetectable and have been reported from <0.2 to 0.7 mg/L.^{94,127} Twenty four hours to 48 h after experimental intra-articular injection of LPS¹²⁷, SAA concentrations in synovial fluid were increased to 100 to 1500 mg/L (>100-fold increase). A systemic response also occurred and was characterized by increased concentrations of SAA in serum. The magnitude of the systemic and

articular responses was dependent on the intra-articular LPS dose. These values decreased promptly to undetectable values when clinical improvement was noticed.

In a study that investigated the concentrations of SAA in serum and synovial fluid from healthy horses and horses with joint diseases, SAA in synovial fluid was found to be increased to high concentrations (>100-fold increase) during septic arthritis compared to concentrations in healthy joints.¹⁸⁹ In this study, ten healthy joints (controls) had low to undetectable systemic and synovial concentrations of SAA; meanwhile four horses with confirmed septic arthritis had moderate to high systemic and synovial SAA concentrations. Also in the same study, two other horses with penetrating wounds had low initial SAA concentrations in synovial fluid, which decreased with joint lavage and anti-inflammatory drugs as well as antimicrobial treatment. The time from injury to sampling in these two latter horses is not reported and this could have influenced the reported low values of SAA, as concentrations of SAA are known to increase at 4 to 8 h in synovial fluid.¹²⁷ In this same study,¹⁸⁹ 3 horses with septic arthritis had undetectable to very low concentrations of SAA in synovial fluid. In two of these 3 horses, synovial fluid samples were only taken after treatment for synovial sepsis was completed (nucleated cell counts were already normal in these two horses when sampling). The third horse received intra-articular corticosteroids 12 days before sampling, which according to the author of the report might have influenced the SAA response. Due to the heterogeneity of the clinical cases reported on the latter study, results are difficult to interpret and a study evaluating synovial SAA concentrations in a large number of confirmed clinical cases of septic arthritis is needed. A promising recent study evaluated the use of SAA concentrations in synovial fluid to differentiate between non-septic arthritis and septic arthritis using a new semi-quantitative colorimetric SAA test (snap test) compared to a quantitative ELISA.¹⁹⁰ The new test had a good specificity (93.3%) and sensitivity (84.6%) showing increased values of synovial SAA in 13 septic synovial samples and low values in 15 non-septic synovial samples.¹⁹⁰ Unfortunately the latter report is currently accessible only as a proceeding abstract and no further details are available.

When the effect of repeated arthrocentesis and repeated intra-articular administration of amikacin on synovial and systemic SAA concentrations were evaluated in healthy equine joints, systemic and synovial concentrations of SAA remained at baseline values.¹⁸⁹ In another study, SAA in synovial fluid was used to evaluate the effect of morphine in horses with experimentally

induced synovitis by intra-articular LPS.¹⁹¹ The concentrations of SAA reduced significantly after intra-articular administration of morphine, compared to control joints.¹⁹¹

2.8 Methods for determination of equine serum amyloid A concentration

Different methods have been used for measurement of SAA in equines. These methods include electroimmunoassay,¹⁹² single radial immunodiffusion,¹¹⁹ enzyme-linked immunosorbent assay (ELISA)¹⁹³ and latex agglutination immunoturbidometric assay.¹⁶³ A commercially available immunoturbidometric assay (LZ test SAA) that was first developed for the measurement of human SAA has been validated in horses.¹⁶⁴ This method has an acceptable intra- and inter-assay variability (1.6% to 24.4% and 4.6% to 33.2%, respectively) with better precision at intermediate and high SAA concentrations compared to low SAA concentrations. The imprecision at low SAA concentrations does not influence the clinical interpretation of results, as horses develop high SAA concentrations (SAA increase from basal values of <30 mg/L to 100 or >1000 mg/L) during the inflammatory response.^{163,164,192,194} The lower limit of quantification of the assay was 0.48 mg/L with a mean of 0.22 mg/L \pm 0.086 when used in serum. A newer study performed an in-house validation of the immunoturbidometric assay in serum and synovial fluid obtaining an intra-assay variability in synovial fluid ranging from 1% to 10% with a lower limit of quantification of 0.05 mg/L; while in serum the intra-assay variability ranged from 0.8% to 16% with a lower limit of quantification of 0.21 mg/L.⁹⁴ This immunoturbidometric assay is automated and fast, and is being used in several diagnostic laboratories in Europe and North America for routine SAA measurements on horse serum and synovial fluid.⁴⁰ It has also been successfully used on serum and synovial fluid in different studies.^{94,127,189,191}

A newer latex agglutination turbidometric immunoassay based on monoclonal anti-human SAA antibodies has been validated for equine, feline and canine use.¹⁹⁵ This test might be associated with a stronger long-term and inter-batch performance.¹⁹⁵ A new semi-quantitative colorimetric SAA test (snap test) has recently been developed and used in synovial fluid from horses.¹⁹⁰ This new test has shown good specificity (93.3%) and sensitivity (84.6%) to diagnose synovial sepsis in horses.¹⁹⁰

All of the methods of quantification found in the literature and used in horses are based on human anti-SAA antibodies and to date no assay specific to equine SAA is available. A pilot study reported an attempt on purifying equine SAA to further develop a specific assay for use in equine.¹⁹⁶ As explained earlier, SAA has a high homogeneity between species and therefore the use of human assays to quantify equine SAA is feasible.

2.9 Other synovial fluid markers used in the diagnosis of septic arthritis

Lactate in normal synovial fluid is 2.02 ± 0.76 mmol/L (range 0.42 to 3.9 mmol/L) and increased to values greater than 4.9 mmol/L in 66% of equine tarsocrural joints after being inoculated with *Staphylococcus aureus*.²⁶ In the latter study, the control joints were injected with saline and lactate remained less than 4.4 mmol/L.²⁶ The pH in synovial fluid can decrease from its normal value of 7.3 ± 0.06 to values below 6.9 during infection and may be of help in diagnosing septic synovitis.^{26,197} Also serum and synovial fluid glucose difference (SSGD) has shown to be useful in diagnosing septic synovitis.²⁶ The normal glucose content in synovial fluid is usually the same or marginally lower than that of serum (SSGD reference value of 0.85 ± 0.59 mmol/L) and the SSGD has shown to increase to values greater than 2.2 mmol/L in 83% of synovial fluid samples from infected joints.²⁶ Although all these parameters are easy and quick to measure, they are not uniformly reliable and therefore it has been suggested in the literature they should not be used as a primary diagnostic parameter.^{26,53}

Nucleated cells are generally predominant during septic synovitis and are the main source of pro-MMP-9.^{21,198} This form of metalloproteinase (measured through gelatin zymography) is increased in synovial fluid from infected joints.^{21,198,199} In one study, septic synovial fluid had higher concentrations of proactive and active MMP-9 compared to non-septic arthritis.¹⁹⁸ Also, the concentration of pro-MMP-9 was predictive of survival as well as the ratio of pro-MMP-9 to pro-MMP-2.²¹ Unfortunately these assays are not easy to run and therefore are not widely available to be used in regular practice.²¹

2.10 Conclusions

The success in treating septic arthritis varies between studies but all of them agree on the importance of appropriate diagnosis, monitoring and treatment. Several diagnostic and prognostic factors have been discussed and all of them should be considered with caution and in parallel with a thorough clinical examination of each clinical case. Treatment for septic synovitis can be aggressive and it can have a large effect on the unspecific synovial markers of inflammation that are traditionally used for diagnosis and monitoring of this condition. As previously mentioned, arthroscopic and through-and-through joint lavages can produce an increase in the synovial concentrations of inflammatory markers to values associated with sepsis. Therefore, the common synovial inflammatory markers should not be used alone and new and more specific markers of synovial infection are needed to diagnose and to monitor sepsis.

The clinical use of serum amyloid A, an acute phase protein very sensitive to inflammation, has recently been investigated in equine medicine. The functions of serum amyloid A are not totally understood and a variety of these were presented within this literature review. The majority of the research found in the literature is based on humans and the number of studies in horses is still low. Elucidation of the functions of SAA within the synovial cavity during sepsis could bring a higher understanding of the pathologic processes involved during infection and possibly, ways to reduce the detrimental effects of septic synovitis. Meanwhile, SAA can increase to high concentrations during septic synovitis in horses and not during other low-inflammatory diseases as reviewed previously; it is not affected by repeated arthrocentesis or repeated intra-articular administration of amikacin. These procedures are commonly performed to diagnose and treat septic synovitis. Further validation of SAA is still needed; and the effect of other common procedures, performed as treatment for septic synovitis, on the concentrations of SAA should be investigated and is the main stem of this thesis. These procedures include two important means of performing lavage of a synovial structure and are arthroscopic lavage and repeated through-and-through joint lavage. Assessing the effect of these invasive procedures in SAA concentrations in synovial fluid will help in the validation of synovial SAA as a marker of synovial sepsis, as well as in the understanding of its unknown functions in the joint during pathologic processes.

CHAPTER 3: EFFECT OF ARTHROSCOPIC LAVAGE ON SYNOVIAL FLUID CONCENTRATIONS OF SERUM AMYLOID A, TOTAL PROTEIN AND NUCLEATED CELL COUNT IN HEALTHY HORSES.

Authors: Andres F. Sanchez-Teran¹, Vet, MSc; José L. Bracamonte¹, DVM, DVSc, Diplomate ACVS & ECVS; Steven Hendrick¹, DVM, DVSc; Hilary J. Burgess¹, DVM, DVSc, Diplomate ACVP; Tanya Duke-Novakovski¹, BVetMed, MSc, Diplomate ACVA & ECVA; Monica Schott¹, DMV; Brent Hoff², DVM, DVSc; and Luis M. Rubio-Martínez³, DVM, PhD, DVSc, Diplomate ACVS, ECVS & ACVSMR.

Institutional affiliations: ¹Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; ²Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, ON, Canada; ³School of Veterinary Science, University of Liverpool, Neston, UK.

Source of funding: Funded by the Equine Health Research Fund of the University of Saskatchewan, Canada (2013).

3.1 Abstract

Objective: To evaluate the effect of arthroscopic lavage on systemic serum amyloid A (SAA) and SAA, total protein, nucleated cell count and percentage of neutrophils in synovial fluid in healthy horses.

Study Design: Prospective, two-period, cross-over study.

Animals: Six healthy horses.

Methods: Middle carpal joints were randomly assigned to one of the following treatments 1) arthrocentesis (controls) or 2) arthroscopic lavage with 30-day washout period between treatments. Synovial fluid and blood samples were collected at 0, 24, 48, 72, 96 and 120 h. Measurements included systemic and synovial fluid SAA, as well as total protein, nucleated cell count and percentages of neutrophils in synovial fluid. Data was analyzed by median quantile regression and Wilcoxon signed-rank test and significance level set at $p < 0.05$.

Results: Systemic and synovial fluid SAA did not increase from baseline (except systemic SAA at 24 h for both treatments) and were not significantly different between treatments. Total protein values were significantly increased after arthroscopic lavage (except at 96 h) but not in controls at all-time points. With both treatments, nucleated cell counts significantly increased from baseline values at all-time points. Percentages of neutrophils were significantly increased after arthroscopic lavage at all-time points whilst only at 24 h in controls.

Conclusions: Total protein, nucleated cell count and percentage of neutrophils values in synovial fluid were significantly increased after arthroscopic lavage, however synovial fluid SAA was not affected by this procedure. Further research is warranted to validate synovial fluid SAA as a monitoring tool during treatment of septic arthritis.

3.2 Introduction

Commonly used synovial fluid parameters for diagnosing and monitoring sepsis in a synovial structure are total protein, nucleated cell count and percentage of neutrophils. The reference values for normal synovial fluid have been documented as a total protein of <25 g/L, nucleated cell count <1 x 10⁹ cells/L and neutrophils being less than 10% of the nucleated cells.^{54,55} Sepsis has typically been considered when synovial fluid yields a total protein higher than 40 g/L, a nucleated cell count above 30 x 10⁹ cells/L and neutrophils comprising greater than 80% of the nucleated cells.^{53,54} However, some authors have suggested synovial sepsis with values of nucleated cell counts $\geq 5 \times 10^9$ cells/L.^{4,8,11,19} Cytological examination and culture of synovial fluid can also aid in the diagnosis of synovial sepsis although bacteria are not always evident on cytology or isolated from cultures.^{11,24} The most prominent cells identified on cytological examination during sepsis are neutrophils, yet degenerative changes are often not present.^{11,24,53}

These synovial fluid parameters can be largely affected by the common procedures performed to treat septic arthritis. A study performed in healthy joints found total protein in synovial fluid to be mildly increased 8 and 14 days post-arthroscopic lavage to values of 27.6 \pm 6.5 g/L and 23.1 \pm 4.9 g/L, respectively.⁷⁹ Additionally, in a recent study performed in healthy joints, total protein peaked at 24 h and nucleated cell count reached values higher than 20 x 10⁹ cells/L at 12 h after arthroscopic lavage with Lactated Ringer's Solution (LRS).⁷⁸ Furthermore, repeated arthrocentesis and repeated intra-articular administration of amikacin every 48 h caused an increase of total protein and nucleated cell count to values in the range of septic arthritis.⁹⁴ Currently, these are the only diagnostic methods available to the majority of equine practitioners. This indicates the need for a new marker that is not affected by the high volume lavage used as part of the treatment for septic arthritis. Subsequently, this marker could accurately diagnose and monitor the response to treatment and establish if further treatments are required.

Serum amyloid A (SAA) is an acute-phase protein that has been investigated as a marker for septic arthritis.¹⁸⁹ Although it is primarily synthesized by the liver, it can also be synthesized by other tissues like synoviocytes, in the presence of infection.¹²⁷ A reference range for systemic SAA concentrations (0.5-20 mg/L) has been reported in healthy horses and those values can have a 100 to 1000-fold increase in response to infectious or inflammatory conditions.^{119,163,194}

Furthermore, systemic SAA concentrations significantly increased in 11 horses that underwent arthroscopic removal of a unilateral osteochondritic lesion of the distal intermediate ridge of the tibia with concentrations that peaked at 48 to 72 h and returned to pre-operative values 5 days after surgery.²⁰⁰ The effect of general anesthesia alone on systemic SAA was evaluated in one small study (including only 2 horses) resulting in no effect on systemic SAA.¹⁹²

Concentrations of SAA in synovial fluid in healthy horses are frequently undetectable and have been reported from <0.2 to 0.7 mg/L.^{94,189} In clinical cases of septic arthritis¹⁸⁹ and 24 to 48 h after experimental intra-articular injection of LPS¹²⁷, synovial fluid SAA concentrations were increased to 100 to 1500 mg/L. These values decreased promptly to undetectable values when clinical improvement was noticed. Moreover, synovial fluid SAA concentrations were not affected by either repeated arthrocentesis or repeated intra-articular administration of amikacin.⁹⁴ This suggests that synovial fluid SAA responds in different magnitude to inflammation than to sepsis, whereas the commonly used synovial fluid parameters total protein, nucleated cell count and percentage of neutrophils may respond identically to non-septic and septic inflammation. However, to the authors' knowledge the effect of arthroscopic lavage on synovial fluid SAA concentrations in horses has not been evaluated. If synovial fluid SAA is not influenced by the high volume lavage performed during arthroscopy, this will suggest it may have a clinical value to be used for monitoring and evaluating sepsis in a joint previously treated by arthroscopic lavage.

The objective of this study was to evaluate the effect of arthroscopic lavage on systemic and synovial fluid SAA concentrations, as well as the effects on total protein, nucleated cell count and percentage of neutrophils in synovial fluid in healthy horses. It was hypothesized that synovial fluid SAA concentrations would not significantly increase from baseline values following arthroscopic lavage, whereas total protein, nucleated cell count and percentage of neutrophils in synovial fluid would increase.

3.3 Materials and Methods

A randomized prospective, two-period, cross-over trial was performed in 6 American Quarter Horses (3 mares and 3 geldings) with a mean age \pm SD of 7 ± 4 years (range 3 to 14 years) and mean \pm SD body weight of 488 ± 29 kg (range 460 to 535 kg). Prior to enrollment,

horses were considered healthy and free of musculoskeletal pathology based on thorough physical examination, lameness examination, and complete blood work (complete blood count, biochemistry profile, and measurement of systemic SAA). Physical and lameness examinations were performed blindly by one investigator (JLB) on each horse every 24 h during the study and 24 h after collection of the last sample. Physical examination entailed: mentation, heart rate, respiratory rate, rectal temperature, mucous membrane color, capillary refill time, and abdominal and thoracic auscultation. Lameness examination was performed at the walk and trot on a straight line on hard surface, before and after flexion of the carpi.

3.3.1 Procedures

For this study, each horse underwent 2 study trials under general anesthesia (control and arthroscopic lavage). Horses and limbs were randomized with a commercial statistics software program (Stata Corp, College Station, TX) to each treatment at the beginning of the study. A 30-day washout period was allowed between treatments; thereafter the contralateral limb received the opposite treatment.

Horses that received arthroscopic lavage were premedicated with xylazine hydrochloride (1mg/kg intravenously IV), induced with ketamine (2 mg/kg IV) mixed with propofol (0.4 mg/kg IV) and maintained with isoflurane. Horses were positioned in dorsal recumbency and the assigned carpus was clipped, aseptically prepared and draped in a routine fashion for arthroscopic surgery. A 3 mL synovial fluid sample of the middle carpal joint was collected prior to arthroscopy using a 22-gauge needle placed into the dorsomedial synovial pouch of the middle carpal joint with the carpus flexed at approximately 70-degrees and labelled as “Time 0”. A 10-mm skin incision was made to create a lateral arthroscopic portal halfway between the extensor carpi radialis tendon and the common digital extensor tendon and midway between the third and intermediate carpal bones with the joint flexed at approximately 70-degrees. A second 10-mm skin incision was then made medial to the extensor carpi radialis tendon and midway between the radial and third carpal bones. The middle carpal joint was then distended with 10 mL of sterile saline using the needle that had been previously inserted to collect the synovial sample. The joint capsule was incised through the lateral skin incision and a 30⁰ - 4.0 mm arthroscope (Richard Wolf GmbH, Knittlingen, Germany) was inserted into the joint as routinely performed

in clinical cases.²⁰¹ Arthroscopic examination of the middle carpal joint was first performed using a fluid irrigation pump (Endomat® SCB n. Hamou®, Karl Storz GmbH & Co. KG, Tuttlingen, Germany). A fluid pump setting of maximal pressure of 100 mmHg and a fluid rate of 300 mL/min were used for initial articular examination. When observed during arthroscopic examination, abnormalities in cartilage, synovial membrane or ligaments were documented and graded.²⁰² A medial portal was made through the dorsomedial skin incision in similar manner to the lateral portal and a 4.5 mm egress cannula (Karl Storz GmbH & Co. KG, Tuttlingen, Germany) was placed and used as an egress portal. The egress cannula was directed as far as possible from the arthroscope and in a different direction to prevent a direct outflow. Copious lavage of the middle carpal joint was performed with a fluid pump setting of 100 mmHg and a fluid rate of 600 mL/min using 10 L of LRS. Skin incisions were closed using 2-0 monofilament polybutester using a cruciate suture pattern. A light sterile bandage consisting of a non-adherent pad, kling gauze and adhesive bandage was then placed to cover the incisions. A blood sample (4 mL) was collected directly from the jugular vein during surgery (time 0 h) by an assistant. Anesthesia and surgery times were recorded. Anesthesia time was defined as the time from delivery of induction drugs to the moment of extubation. Surgical time was defined as the time from creation of the first incision to closure of arthroscopic portals. Horses recovered unassisted in a padded recovery stall. Skin sutures were removed two weeks after arthroscopy.

Control horses were placed under general anesthesia with the same anesthetic protocol and the assigned carpus was clipped and aseptically prepared in a similar fashion. Arthrocentesis of the middle carpal joint was performed to collect 3 mL of synovial fluid as previously described and labelled as “time 0 h”. A blood sample (4 mL) was collected directly from the jugular vein at the same time. Anesthesia time was recorded and horses were kept under general anesthesia for 90 minutes as an estimated time required to perform arthroscopic lavage in clinical cases. A light sterile bandage was applied on the treated joint in the same manner as on the limbs that received arthroscopic lavage and horses recovered as described previously.

3.3.2 Postoperative sampling and monitoring

Physical and lameness examinations were performed blindly in both groups and as previously described at 24, 48, 72, 96 and 120 h after general anesthesia. After each examination

horses were sedated with detomidine (0.005 to 0.006 mg/kg IV), bandages were removed and synovial fluid samples (3 mL) were collected from the middle carpal joint by one of the investigators (AST). Sterile technique was used to place a 22-gauge needle into the dorsolateral synovial pouch of the assigned middle carpal joint. The number of needle replacements required to obtain a synovial fluid sample was recorded each time. The same investigator who performed the arthrocenteses (not blinded to treatment distribution) documented subjectively heat, pain and joint effusion as mild, moderate or severe. A blood sample was collected from the jugular vein at the same time points as arthrocenteses.

3.3.3 Sample analysis

Synovial fluid samples were assessed macroscopically by one of the investigators (JLB) who was blinded to treatment distribution. Blood contamination was subjectively classified on a scale from 0 to 4 as follows: 0) clear with no signs of blood contamination, 1) one drop of blood or hemorrhagic color at needle-end of syringe, 2) mildly hemorrhagic, slightly uniform red color, 3) moderately hemorrhagic, uniform red color but still translucent and 4) severely hemorrhagic, uniformly red not translucent. Total protein in synovial fluid was measured immediately after collection directly from the syringe using a standard refractometer (Reichert GmbH, Seefeld, Germany). Samples were then transferred into two EDTA tubes (1.5 mL each).

A single board certified clinical pathologist (HJB) who was blinded to treatments, examined one aliquot of the synovial fluid sample. Subjective assessment of color, clarity and presence or absence of solid material was performed. A direct smear was made before the sample was treated with hyaluronidase (hyaluronidase from bovine testes; type VIII lyophilized powder 300- 1000 U/mg; SIGMA[®], St. Louis, MO). Nucleated cell counts were obtained using an automated hematology analyzer (Abbott Laboratories, IL) and a cytocentrifuge preparation (Shandon Southern Instruments Inc., PA) was prepared for each sample. All slides were stained using a Romanowsky stain (Fisher Scientific Company L.L.C., Middletown, VA). Cytology slides were subjectively assessed for cellular preservation, degree of hemodilution and presence of etiologic agents. A 100 cell differential count was performed on each sample using the cytocentrifuge preparation.

The other aliquot of synovial fluid and the blood samples were centrifuged at 2100 g for 15 minutes (Beckman Coulter Inc., Germany). Synovial fluid supernatant and serum were stored at -80 °C for further SAA quantification. Quantification of systemic and synovial fluid SAA was performed using a human SAA turbidometric immunoassay (Eiken Chemical Co., Tokyo, Japan) previously validated for use in equine¹⁶⁴ and used on previous studies on equine synovial fluid.^{94,127,189} Previous to quantification of SAA by an automated chemistry analyser (F. Hoffmann-La Roche Ltd, Basel, Switzerland), 10 uL of hyaluronidase (SIGMA®; 500 U/mL, prod. No. H4272, lot SLBB1402V) were added to 490 uL of synovial fluid to reduce synovial fluid viscosity.

3.3.4 Statistical analysis

All data was analyzed with a commercial statistics software program (Stata 12, StataCorp, College Station, TX, USA). Descriptive statistics (means, standard deviations, medians, percentiles, stem and leaf plots, quantile plots and histograms) were used to summarize the distribution and central tendency of the independent and dependent variables. Normality of data was assessed using the Shapiro-Wilk test and variables with $p < 0.05$ were interpreted as not normally distributed. As many of the outcomes measured were not normally distributed, non-parametric statistics were used for evaluation. The outcomes compared included total protein, nucleated cell count, percentage of neutrophils, systemic SAA and synovial fluid SAA concentrations. Values of SAA that were below the lower limit of quantification of the assay were interpreted as “0” (zero) for statistical analysis. Quantile regression was used to model the median value of each of the outcomes in separate models with treatment, time and an interaction term (treatment x time). The treatment medians were separated by time using the Wilcoxon signed-rank test. Significance level was set at $p < 0.05$.

3.4 Results

All horses remained healthy and sound throughout the entire study. The mean \pm SD anesthesia time for horses undergoing arthroscopic lavage was 72.6 ± 17.2 minutes and for controls was 85.3 ± 7.84 minutes. Mean \pm SD surgical time to infuse 10 L of LRS was 24 ± 2.1

minutes. Mild synovial fluid effusion of the middle carpal joint was noticed after arthroscopic lavage. The effusion was not associated with heat or pain on palpation and resolved within 5 days without further treatment. One carpus developed mild effusion of the extensor carpi radialis tendon sheath after arthroscopic lavage with no associated pain, heat or lameness. This resolved itself within 4 days without medical treatment. In all carpi explored by arthroscopy, no abnormalities were noticed in cartilage, synovial membrane or ligaments except for one. This latter carpus had grade 2 cartilage lesions extending down to <50% of cartilage depth²⁰² with change in color and ‘wear lines’ in the cartilage surface, increased synovial villi density and thickening. No osteochondral fragments, fractures or ligaments abnormalities were observed. Both carpi in this horse had values considered normal on all measured synovial fluid markers at the beginning of the study.

Synovial fluid samples from control carpi were clear with no evidence of contamination from peripheral blood at all sampling times except from two horses. One of these had blood contamination at only one sampling time (72h; score 2). The second horse had blood contamination at various sampling times graded as score 1 at 0, 72 and 96 h and score 3 at 24 and 48 h. Twenty-four hours after arthroscopic lavage, all synovial fluid samples were scored as 4 and were turbid and very viscous, improving progressively to score 3 at 96 h. By the end of the study (120 h), all samples from the arthroscopic lavage treated carpi improved to score 2 and were amber in color, slightly cloudy and slightly viscous except for samples from one carpus which remained severely hemorrhagic during all sampling times (score 4). The former was the only carpus from the arthroscopic lavage treatment in which needle replacement was performed (at 3 sampling times). Only one horse in the control group had the needle replaced and at one sampling time point.

3.4.1 Protein concentrations in synovial fluid

Figure 1 summarizes synovial fluid total protein values. Baseline values were not significantly different between treatments. Overall total protein median of arthroscopic lavage treatment was significantly higher than controls’ median ($p=0.01$).

Total protein values after arthroscopic lavage (all time points except baseline) ranged from 20.0 to 61.0 g/L with a median of 34.5 g/L. Total protein values significantly increased

($p<0.05$) 24 h after the procedure and remained increased ($p<0.05$) until the end of the study (except at 96 h). In the control carpi, after the first arthrocentesis, total protein values (overall of all times except baseline) ranged from 10.0 to 42.0 g/L with a median of 28.0 g/L and did not significantly increase compared to baseline values. When median values at each sampling time were compared between treatments, total protein value was significantly higher after arthroscopic lavage than in controls only at 24 h ($p<0.05$).

3.4.2 Nucleated cell count in synovial fluid

Figure 2 summarizes synovial fluid nucleated cell count values. Baseline values were not significantly different between treatments. Overall, nucleated cell count medians were not significantly different between groups.

Nucleated cell count values ranged from 0.4 to 23.3×10^9 cells/L with a median of 1.5×10^9 cells/L after arthroscopic lavage (overall of all times except baseline) and from 0.2 to 7.1×10^9 cells/L with a median of 1.1×10^9 cells/L after the first arthrocentesis (overall of all times except baseline). In both groups, nucleated cell count significantly increased at all time points when compared to baseline values ($p<0.05$). After arthroscopic lavage the nucleated cell count was significantly higher than controls only at 24 h ($p<0.05$).

3.4.3 Percentage of neutrophils in synovial fluid

Figure 3 summarizes the percentage of neutrophils obtained. Baseline values were significantly different between treatments ($p=0.03$). There was no significant difference between treatments when comparing their overall percentage of neutrophils medians.

Percentage of neutrophils values after arthroscopic lavage (overall of all times except baseline) ranged from 4% to 88% with a median of 49.5% and significantly increased 24 h after arthroscopic lavage ($p<0.05$). Values remained significantly increased after arthroscopic lavage until the end of the study compared to baseline values. In the control carpi, percentages of neutrophils ranged from 3% to 76% with a median of 39.5% (overall of all times except baseline) and were only significantly increased from baseline values at 24 h ($p<0.05$). When

median values at each sampling time (excluding baseline values) were compared between treatments, there was no significant difference between groups.

3.4.4 Serum amyloid A in synovial fluid

Figure 4 summarizes synovial fluid SAA values. Baseline values were not significantly different between treatments. There was no significant difference between treatments when comparing their overall synovial fluid SAA medians.

Synovial fluid SAA (overall of all times except baseline) ranged from less than the lower limit of quantification (LOQ) of the assay (0.2 mg/L)^{94,164} to 1.3 mg/L with a median of <LOQ after arthroscopic lavage and from <LOQ to 4 mg/L with a median of <LOQ after the first arthrocentesis. Two control horses had values higher than values seen in normal horses in previous studies^{94,189} at 48 h (1.3 mg/L and 4 mg/L), as well as one horse 24 h after arthroscopic lavage (1.3 mg/L). The values obtained on these 3 horses are represented as outliers in Figure 4. In both groups, synovial fluid SAA did not significantly increase from baseline values. There was no significant difference when values of synovial fluid SAA at each sampling time were compared between treatments.

3.4.5 Systemic serum amyloid A

Figure 5 summarizes systemic SAA values. Baseline values were not significantly different between treatments. There was no significant difference between groups when comparing their overall systemic SAA medians.

Systemic SAA values (overall of all times except baseline) ranged from <LOQ to 92.7 mg/L with a median of 0.4 mg/L after arthroscopic lavage and from <LOQ to 243.7 mg/L with a median of 0.3 mg/L after the first arthrocentesis. Two control horses had a maximal increase of systemic SAA (130.8 mg/L and 243.7 mg/L) at 48 h. One treated horse had a maximal increase of systemic SAA 24 h after arthroscopic lavage (92.7 mg/L). These 3 horses were the same horses reported to have a mild increase on synovial fluid SAA and their SAA values are represented as outliers in Figure 5. Systemic SAA values in both groups significantly increased

compared to baseline values only at 24 h ($p < 0.05$). There was no significant difference when values at each sampling time were compared between treatments.

3.5 Discussion

Arthroscopic lavage with copious fluids is an important aspect in the treatment of septic arthritis.⁴ In our study synovial fluid SAA was not affected by this procedure while total protein, nucleated cell count and percentage of neutrophils values were all increased when compared to baseline values. These findings, supported by the fact that synovial fluid SAA increases to high concentration during septic arthritis¹⁸⁹, suggest that synovial fluid SAA may be valuable for monitoring the response of septic joints treated by arthroscopic lavage. Further studies assessing synovial fluid SAA as a monitoring tool during treatment of septic arthritis are needed and should include sequential measurements of synovial fluid SAA in clinical cases.

Carpi that received arthroscopic lavage had a significant increase in total protein, nucleated cell counts and percentages of neutrophils in synovial fluid. Most total protein values at 24 h were within the reported range for septic arthritis (≥ 40 g/L).^{54,55} In addition, significant increase in the values of nucleated cell counts up to 23.3×10^9 cells/L were observed at 24 h after arthroscopic lavage which can be considered consistent with sepsis. Furthermore, median values of percentages of neutrophils were significantly increased after arthroscopic lavage and some values were within the reported range for septic arthritis ($\geq 80\%$).^{4,8} Values of a similar magnitude were obtained in a recent study in healthy tarsocrural joints after being distended, followed with lavage with LRS for 30 minutes.⁷⁸ An earlier experimental study reported significant changes in synovial fluid markers in normal joints at days 8, 14, 21 and 28 after arthroscopic lavage and after arthroscopic partial synovectomy.⁷⁹ Direct comparison with their results cannot be made as in the previous study, samples were taken 8 days post-operatively and the immediate inflammatory response after arthroscopy was missed. Nevertheless, clinicians should be aware that the commonly used synovial fluid markers to evaluate sepsis are nonspecific as they can significantly increase after arthroscopic lavage in a manner similar to synovial sepsis. Therefore, their use to monitor the progression of sepsis postoperatively can be challenging.

The effect of repeated arthrocentesis on synovial fluid markers has been evaluated in previous studies with similar results.^{94,203} In our study, values of total protein, nucleated cell counts and percentages of neutrophils in control carpi remained below the values considered for septic arthritis except for one total protein value obtained at 96 h (42 g/L). This higher value could be due to an individual response to repeated arthrocentesis. Although our results are similar to a recent study where arthrocentesis was performed every 48 h⁹⁴, total protein in two control carpi in our study were higher than the published normal value (<25 g/L)^{54,55} at 0 h. These two total protein values and the difference found between groups in the percentage of neutrophils at the beginning of the study could be an effect of blood contamination when sampling or a sub-clinical synovitis that was not detected during our physical and lameness examination at the beginning of the study. Our values for total protein, nucleated cell counts and percentages of neutrophils in control carpi are very similar to those of a previous report in tendon sheaths after more frequent synovial centesis with one single injection of LRS.²⁰³ However, another study reported a significant increase in total protein after repeated arthrocentesis with intra-articular injection of saline solution instilled at each arthrocentesis.¹⁸⁹ These differences in total protein values between studies may be associated with different study designs.

During arthroscopic lavage of clinical cases of septic arthritis, two or more egress portals may be used in an attempt to achieve thorough removal of fibrin or pannus, especially if present in the palmar synovial pouches.⁴¹ One single egress portal was used in this study and precautions were taken to avoid direct flow between the inflow and outflow of the lavage solution by placing the arthroscope (ingress) far from the egress cannula and directing both in different directions within the joint. The authors believe this was sufficient to create a thorough lavage of the joint as these were all healthy joints and no pannus or fibrin was present during the lavage.

One carpus developed distension of the extensor carpi radialis tendon sheath possibly by inadvertent penetration of the sheath during creation of the portals. The change in synovial fluid markers found in this carpus is similar to the rest of carpi that received arthroscopic lavage and therefore the distension encountered in the tendon sheath had no effect on our results.

One carpus had signs of cartilage degeneration observed during arthroscopic examination. The contralateral carpus acted as a control and the synovial fluid markers behaved similar to the other carpi that served as controls. The authors believe that the significant changes in the synovial markers in this specific carpus after arthroscopic lavage can be accounted to the

procedure and not to the pathological cartilage degeneration itself. Interestingly, neither systemic nor synovial fluid SAA in either carpus were elevated. This correlates with a previous report of SAA levels in horses with osteoarthritis and osteochondrosis.¹⁸⁹

Systemic SAA significantly increased at 24 h in both groups. A moderate increase in 3 horses after general anesthesia (2 controls and 1 after arthroscopic lavage) to values up to 243.7 mg/L, could be responsible for this statistical significance. The cause for this increase is unknown. It is suspected it could be associated to sub-clinical conditions aggravated by anesthesia, procedures related to anesthesia (catheter placement, intubation, prolonged recumbency) or to anesthesia itself which can cause a mild transient respiratory inflammatory response.²⁰⁴ Previous research has shown significant increase of systemic SAA values after elective arthroscopic removal of an osteochondritic lesion.²⁰⁰ However, 2 of the 3 horses that had increased systemic SAA values in our study were controls and consequently only arthrocentesis was performed under general anesthesia. This disagrees with a previous small study performed on 2 horses where general anesthesia alone had no effect on systemic SAA.¹⁹² The present study was not designed to evaluate the effect of anesthesia alone on systemic SAA; therefore further studies on the effect of anesthesia on systemic SAA are warranted.

Portal creation and capillary lesions of the highly vascularized synovial membrane during arthroscopic lavage could be responsible for the synovial samples graded as score 4 encountered 24 h after arthroscopic lavage. The presence of blood in the joint itself can be partially responsible for the increase in synovial fluid markers and can also cause further inflammation increasing total protein and neutrophil concentrations.²⁰⁵ In our study, all synovial fluid samples from the arthroscopic lavage treated carpi were scored as 4 (according to our blood contamination scoring) at 24 h after arthroscopic lavage. The samples improved progressively to a score 3 by 96 h and by the end of the study almost all samples improved to a score 2. This finding is supported by a recent study in which samples were found to be red 12 h after arthroscopic lavage with LRS and remained red until the end of the study (48 h).⁷⁸ Furthermore, in another study, samples collected at 8, 14, 21 and 28 days after arthroscopic lavage were found to be slightly hemorrhagic even at 28 days.⁷⁹

The effect of blood contamination on synovial fluid SAA concentration cannot be evaluated in this study as all horses had low concentrations of systemic SAA. The 3 horses reported in this study that had a moderate increase in their systemic SAA after general anesthesia

showed a mild (not statistically significant) increase in synovial fluid SAA to values higher than values seen in normal horses in previous studies.^{94,189} Serum amyloid A isoforms from serum were detected in synovial fluid in a previous study.¹²⁷ Therefore, the authors suspect the increase in synovial fluid SAA found in these 3 horses could derive from systemic SAA infiltration into the joint by either direct blood contamination or by capillary diffusion. However, the effect of contamination of synovial fluid with systemic SAA is still unclear and further studies should be performed to evaluate its effect on synovial fluid SAA quantification. Meanwhile, caution should be taken when interpreting synovial fluid SAA values from horses with systemic inflammation (e.g. foals with septicemia).

Arthroscopic lavage altered the common synovial fluid markers and in some cases these markers reached the cut-off value for sepsis. These synovial fluid markers seem to respond in the same manner to non-septic and septic inflammation, which could complicate evaluation of a joint for sepsis while treatment is installed. Synovial fluid SAA was not affected in either treatment groups in this study and is known to increase 100 to 1000-fold from its normal concentrations during sepsis.¹⁸⁹ Therefore, SAA may serve as a more accurate marker for monitoring sepsis than total protein, nucleated cell count and percentage of neutrophils when measured in synovial fluid from horses that have undergone arthroscopic lavage. Further research in clinical cases is still needed before recommending synovial fluid SAA as a monitoring tool during treatment for septic arthritis. Furthermore, caution should be taken when interpreting synovial fluid SAA from horses with systemic inflammation until further research is available. Additional evaluation of synovial SAA as a marker of sepsis in clinical cases after arthrocentesis and arthroscopic lavage is warranted.

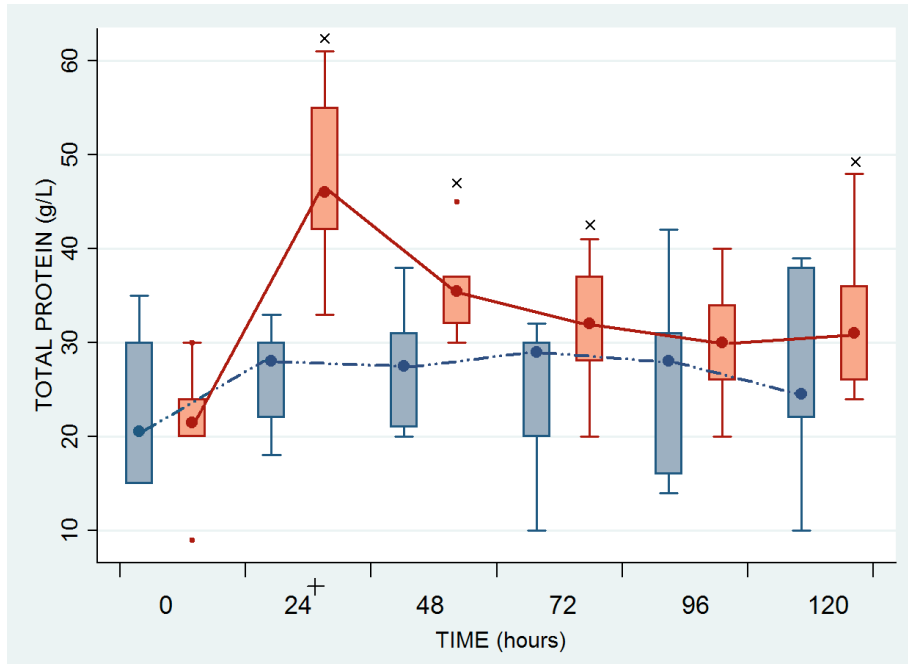


Figure 1. Box plot of median (circles) total protein concentrations (g/L) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference ($p < 0.05$) from baseline. ⁺Significant difference ($p < 0.05$) between treatments.

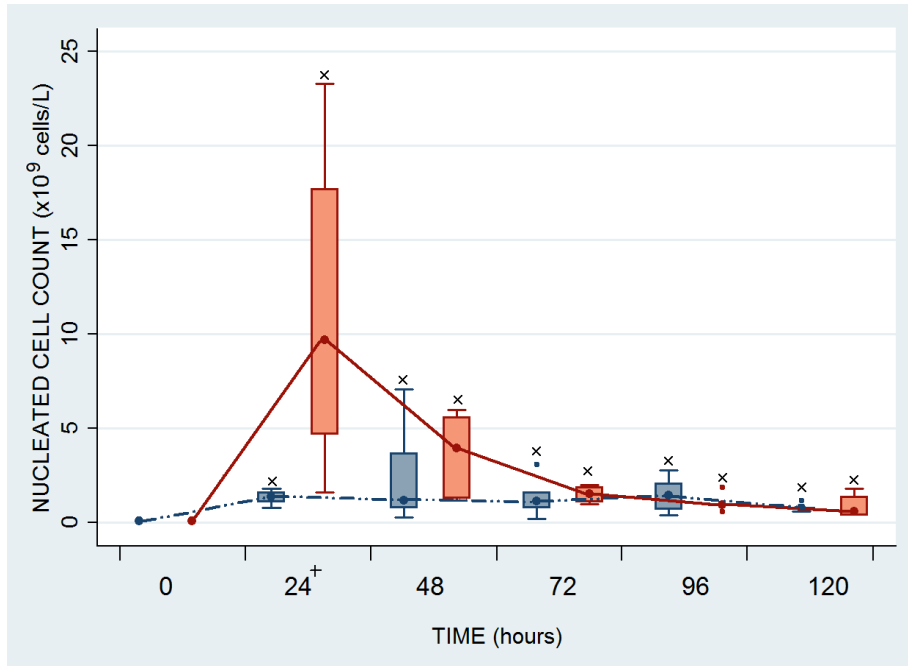


Figure 2. Box plot of median (circles) nucleated cell counts ($\times 10^9$ cell/L) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference ($p < 0.05$) from baseline. ⁺Significant difference ($p < 0.05$) between treatments.

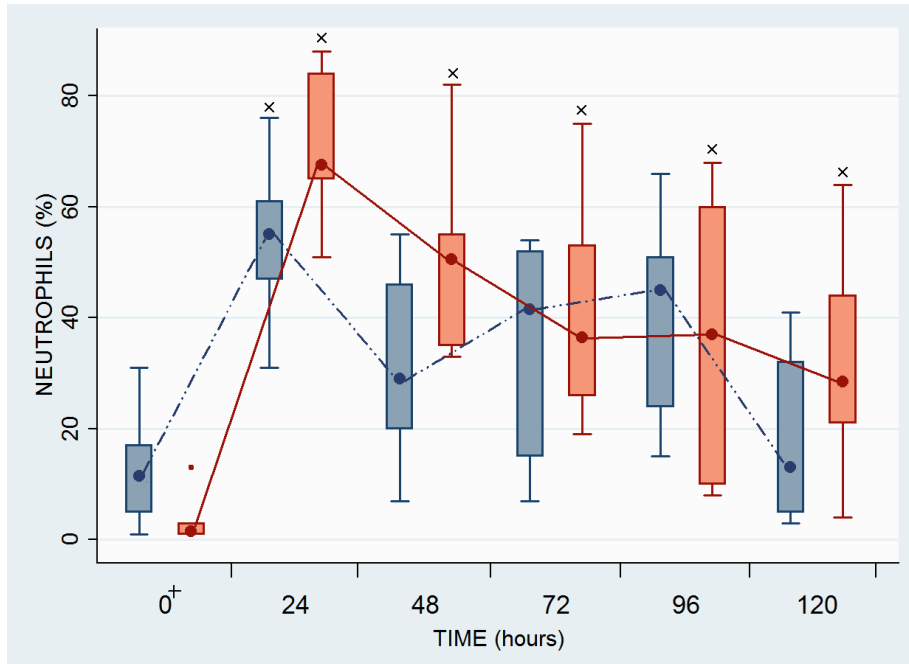


Figure 3. Box plot of median (circles) percentage of neutrophils (%) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference ($p < 0.05$) from baseline. ⁺Significant difference ($p < 0.05$) between treatments.

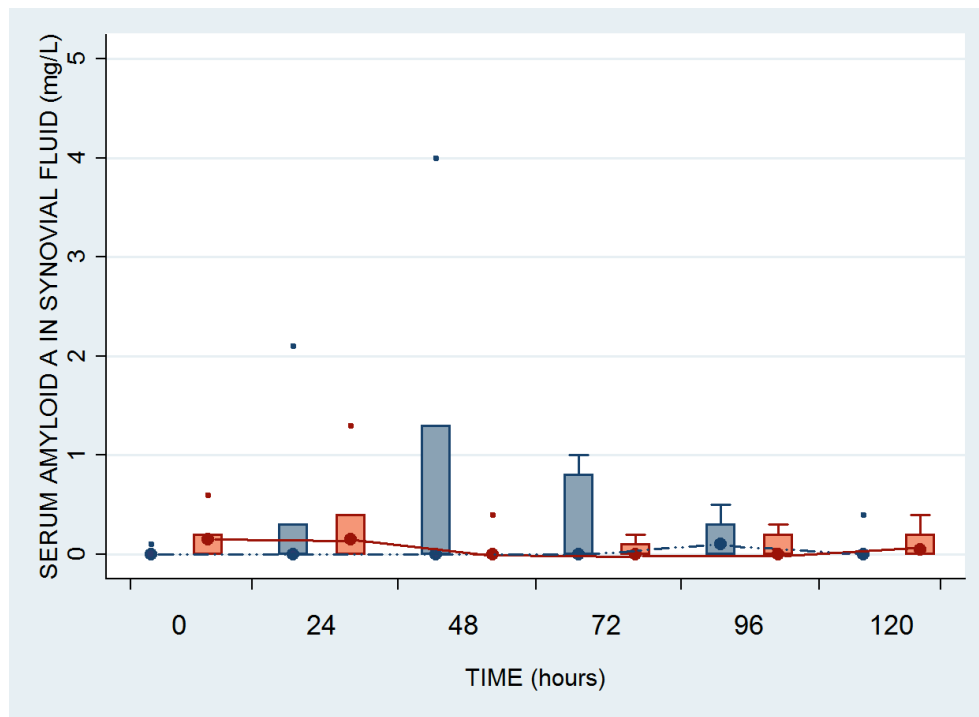


Figure 4. Box plot of median (circles) SAA concentrations (mg/L) in synovial fluid from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^XSignificant difference ($p < 0.05$) from baseline. ⁺Significant difference ($p < 0.05$) between treatments.

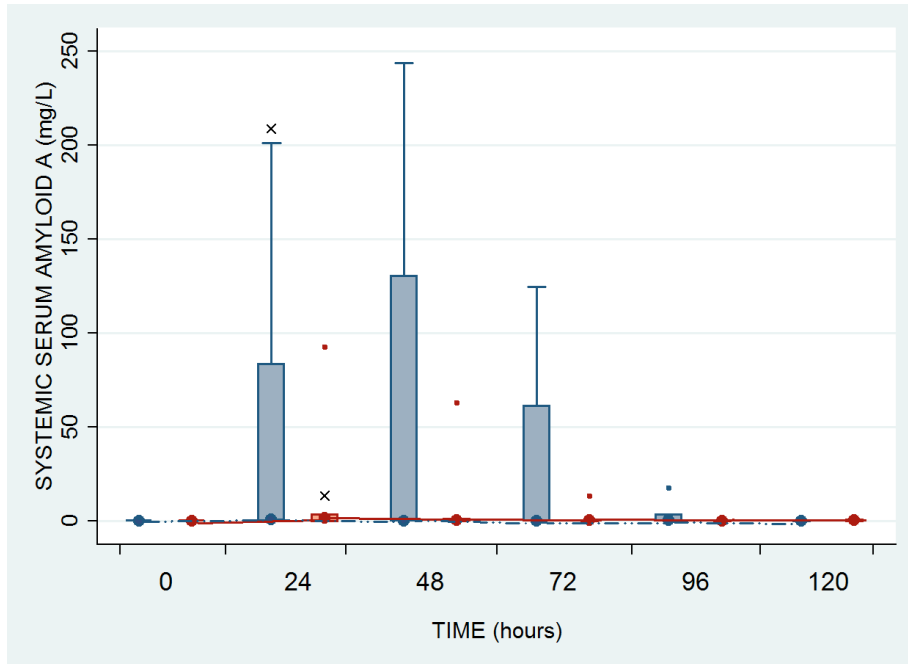


Figure 5. Box plot of median (circles) systemic SAA concentrations (mg/L) from control middle carpal joints (dotted line; blue) and middle carpal joints after arthroscopic lavage (solid line; red) in healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ■ Outliers. ^xSignificant difference (p<0.05) from baseline. ⁺Significant difference (p<0.05) between treatments.

CHAPTER 4: EFFECT OF REPEATED THROUGH-AND-THROUGH JOINT LAVAGE ON SERUM AMYLOID A IN SYNOVIAL FLUID FROM HEALTHY JOINTS.

Andres F. Sanchez-Teran¹, Vet, MSc; José L. Bracamonte¹, DVM, DVSc, Diplomate ACVS & ECVS; Steven Hendrick¹, DVM, DVSc; Lea Riddell¹, BSc; Kristyna Musil¹, DVM, MVetSc; Brent Hoff², DVM, DVSc; and Luis M. Rubio-Martínez³, DVM, PhD, DVSc, Diplomate ACVS, ECVS &ACVSMR.

Institutional affiliations: ¹Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; ²Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, ON, Canada; ³School of Veterinary Science, University of Liverpool, Neston, UK.

Source of funding: Funded by the Equine Health Research Fund of the University of Saskatchewan, Canada (2014).

4.1 Abstract

The objective of this study was to evaluate the effect of through-and-through joint lavage on systemic and synovial SAA; as well as total protein, nucleated cell count and percentage of neutrophils in synovial fluid of 6 healthy horses. A prospective experimental study was performed where one tarsocrural joint of each horse was randomly assigned to receive repeated through-and-through joint lavage at 0, 48 and 96 h. Synovial fluid and blood samples were collected at 0 (baseline), 24, 48, 72, 96 and 120 h. Systemic and synovial SAA, total protein, nucleated cell count and percentage of neutrophils were measured and compared to baseline. Data was analyzed using quantile regression and significance level set at $p < 0.05$. Concentrations of systemic and synovial SAA were not increased from baseline. Total protein and nucleated cell counts were significantly increased from baseline (except for nucleated cell count at 96 h); and percentage of neutrophils values were not significantly increased. Repeated through-and-through joint lavage did not affect synovial SAA concentrations in horses, however synovial total protein and nucleated cell counts values increased. Some of the total protein and nucleated cell counts values observed in this study were within the range reported for septic arthritis 24 h after joint lavage. Synovial SAA may be a valuable marker to be used when evaluating the clinical progression of septic joints after through-and-through joint lavage. Clinical studies evaluating synovial fluid SAA concentrations while treating synovial sepsis with through-and-through joint lavage are warranted.

4.2 Introduction

Septic arthritis is a severe arthropathy with survival rates ranging from 62% in foals to 85% in adult horses¹¹ and only 48.3% to 65.8%^{206,207} of the horses that survive will regain athletic function. Treatment can be performed in various modalities including arthroscopic lavage¹⁹ and through-and-through joint lavage of the affected synovial structure.⁵⁷ These procedures are usually combined with administration of antimicrobials by intra-articular injections or by intravenous regional limb perfusion.⁸⁷ The preferred procedure to lavage an infected joint is by arthroscopy.¹⁹ However, in many occasions because of economical constraints or anesthetic risk, septic joints are successfully treated by repeated through-and-

through joint lavage.⁵⁷ Because of the limited debridement and the lower volume of lavage fluids that through-and-through joint lavage allows in comparison with arthroscopy; through-and-through joint lavage is usually repeated to increase the likelihood of clinical success.⁵⁷

Bacteria are not always evident on cytology or isolated from cultures¹¹ and therefore diagnosis of synovial sepsis is usually relied upon total protein, nucleated cell count and percentage of neutrophils in synovial fluid. Reference values in normal synovial fluid have been established as total protein of <25 g/L, nucleated cell count <1 x 10⁹ cells/L and neutrophils being less than 10% of the total nucleated cells.⁵⁴ Sepsis has typically been considered with total protein higher than 40 g/L, nucleated cell count above 30 x 10⁹ cells/L and neutrophils comprising greater than 80% of the total nucleated cells.⁵⁴ However, other authors have suggested synovial sepsis with values of nucleated cell count $\geq 5 \times 10^9$ cells/L.^{11,19}

Practitioners usually rely on sequential cytological examination of synovial fluid samples as a guideline for therapy adjustments and prognosis.^{12,57} Unfortunately, repeated intra-articular administration of antimicrobials,⁹⁴ repeated arthrocentesis,²⁰³ arthroscopic lavage²⁰⁸ and a single through-and-through joint lavage^{80,82,83} can result in similar cytological changes as those associated with septic arthritis. Therefore, common synovial fluid parameters are not reliable especially after the joint has been treated.

Serum amyloid A in synovial fluid increases from undetectable or very low concentrations in healthy joints (<0.2 to 0.7 mg/L),^{94,189} to high concentrations during synovial sepsis (100-1500 mg/L).¹⁸⁹ Its concentrations are not affected by repeated arthrocenteses,^{94,189} intra-articular administration of amikacin⁹⁴ or arthroscopic lavage.²⁰⁸ A single through-and-through joint lavage has been reported to affect the common inflammatory markers.^{80,82,83} However, the effect of repeated through-and-through joint lavage on these markers and on synovial SAA concentration has not been investigated.

The objective of this study was to evaluate the effect of repeated through-and-through joint lavage on systemic and synovial SAA concentrations; as well as total protein concentration, nucleated cell count and percentage of neutrophils in synovial fluid from healthy equine tarsocrural joints. We hypothesized that synovial SAA will not change from baseline values after repeated through-and-through joint lavage while the other synovial parameters will increase significantly.

4.3 Material and Methods

Six healthy adult American Quarter Horses (7 ± 3.5 years; 525 ± 64 kg b.w.t.) were used for this prospective experimental study. The University Animal Care and Use Committee approved all experimental procedures. Horses were considered healthy and free of musculoskeletal pathology on the basis of results of a thorough physical examination, lameness examination, complete blood work (CBC and biochemistry profile including measurement of systemic blood SAA) and radiographic assessment of the tarsi (4 standard views).

A tarsocrural joint in each horse was randomly assigned by a commercial statistics software program (Stata Corp.) to receive repeated through-and-through joint lavage at times 0, 48 and 96 h. Samples of synovial fluid were taken immediately before performing the joint lavage and at times 24, 72 and 120 h. Synovial fluid parameters measured at all time points included: total protein, nucleated cell count, percentage of neutrophils and synovial SAA. A blood sample was collected in a serum tube by jugular venipuncture at all time points before arthrocentesis was performed. Serum and synovial fluid samples taken at 0 h were used as baseline values and served as controls for further statistical analysis. Physical and lameness examinations were performed every 24 h on all horses until the last sampling (120 h) and 7 days after the third lavage.

Horses were restrained in stocks and sedated with detomidine hydrochloride (0.02 mg/kg IV) and butorphanol tartrate (0.03 mg/kg IV). The assigned tarsus was clipped and aseptically prepared. Prior to joint lavage, 3 mL of synovial fluid was collected from the dorsomedial pouch of the tarsocrural joint using a 22G, 3.8 cm long needle.²⁰⁹ The sample was then separated in two aliquots (1.5 mL each) and placed into two EDTA tubes. The number of attempts to obtain a synovial fluid sample (needle replacements) was recorded, as well as any complication found while lavage was performed.

Through-and-through lavage of the tarsocrural joint was performed after synovial collection using a 14G, 3.8 cm long needle, inserted perpendicular to the skin into the dorsomedial pouch, medial to the saphenous vein and approximately 2.5 cm distal to the level of the palpable medial malleolus.²⁰⁹ A second 14G, 3.8 cm long needle was then inserted perpendicular to the skin into the dorsolateral pouch of the tarsocrural joint lateral to the extensor tendons just below the palpable lateral malleolus of the tibia. Sterile pump tubing was attached to

the needle inserted in the dorsomedial joint pouch and 3 L of LRS were infused using a peristaltic fluid pump (Spencer Varistaltic Dispenser and Pump, Manostat) at a flow setting of 150 mL/min. After lavage was performed, excess fluid was evacuated from the joint before retrieving the needles. Light bandages consisting of sterile non-adherent pad (Medline Industries Inc.), kling gauze (Covidien) and adhesive bandage (BSN Medical) were applied.

4.3.1 Sample Analysis

A single clinical pathologist, who was blinded to times of collection and study protocol, examined one aliquot of the synovial fluid sample. Subjective assessment of color, clarity and presence or absence of solid material was performed. Samples were subjectively graded for blood contamination in a blinded fashion according to a scale from 0 to 4 as follows: 0) clear with no signs of blood contamination, 1) slightly uniform hemorrhagic color, 2) mildly hemorrhagic, mild uniform red color, 3) moderately hemorrhagic, uniform red color but still translucent or 4) severely hemorrhagic, uniformly red and not translucent. Total protein was determined through refractometry (Reichert GmbH). A direct smear was made before the sample was treated with 0.1 mg of hyaluronidase (hyaluronidase from bovine testes; type VIII lyophilized powder 300 to 1000 U/mg; SIGMA). Nucleated cell count was obtained using an automated hematology analyzer (Abbott Laboratories). A cytocentrifuge preparation (Shandon Southern Instruments Inc.) was prepared and stained using a Romanowsky stain (Fisher Scientific Company L.L.C.). Cytological examination was subjectively assessed for cellular preservation and presence of etiologic agents. A 100 cell differential count was performed on each sample using the cytocentrifuge preparation.

The second aliquot of synovial fluid and blood samples were centrifuged at 2100 g for 15 minutes (Beckman Coulter Inc.). Synovial fluid supernatant and serum were stored at -80 °C. When collection of all samples was finalized, synovial fluid and serum samples were thawed at room temperature. Ten µL of hyaluronidase (SIGMA; 500 U/mL) were added to 490 µL of synovial fluid to reduce its viscosity. Quantification of systemic and synovial SAA was performed on an automated chemistry analyzer (F. Hoffmann-La Roche Ltd) using a human SAA turbidometric immunoassay (Eiken Chemical Co.) previously validated for equine use.¹⁶⁴

4.3.2 Statistical Analysis

All data was analyzed with a commercial statistics software program (Stata 12IC, Stata Corp.). Descriptive statistics were used to summarize the distribution and central tendency of the independent and dependent variables. Many of the measured outcomes were not normally distributed and non-parametric statistics were used for evaluation. Quantile regression was used to compare the baseline median value of each of the outcomes at each sampling time point in separate models. The outcomes included total protein, nucleated cell count, percentage of neutrophils, systemic SAA and synovial SAA concentrations. The degree of blood contamination and needle replacements were individually tested as potential confounding variables for all outcomes in the quantile regression models. The overall significance of each confounding variable in the models was tested with an F-test. Significance level was set at $p < 0.05$.

4.4 Results

All horses remained healthy and did not show lameness throughout the study. Joint lavage was performed successfully in all horses with minor complications. These included movement of the horse during the procedure, which resulted in flexion of the limb and short interruption of the egress flow. The mean \pm SD time to infuse 3 L of LRS was 23 ± 3 minutes. Mild effusion of the treated tarsocrural joint was noticed after the first lavage in all horses and resolved within seven days after performing the last joint lavage. The effusion was not associated with heat or pain on palpation.

When obtaining synovial fluid samples, needle replacement with a new 22G needle occurred in 9 out of 36 samples because of movement of the horse when sampling or insufficient synovial fluid quantity collected. When needle replacement was evaluated as a confounding variable it was found to have no influence on the outcomes. Baseline samples were subjectively scored according to the amount of blood contamination as grade 4 in two samples, grade 2 in one sample, grade 1 in one sample and grade 0 in two samples. In the rest of the sampling times (not including baseline), blood contamination was subjectively graded 4 in seven samples, 3 in eleven samples, 2 in eleven samples, and 0 in one sample. Blood contamination score was found to be a

confounding variable only to percentage of neutrophils ($r^2 = 0.44$, $P < 0.001$) and a higher blood contamination score indicated a higher neutrophil count.

Total protein baseline values ranged from 10.0 to 25.0 g/L with a median of 12.5 g/L. Once repeated joint lavage was initiated, total protein values ranged from 15 to 60 g/L with a median of 26.0 g/L throughout the study. Concentrations of total protein peaked at 24 h after the first lavage and remained significantly increased compared to baseline at all time points (Figure 6).

Nucleated cell counts followed a similar pattern to the one reported for total protein. Baseline values ranged from 0.1 to 0.5 x 10⁹ cells/L with a median of 0.3 x 10⁹ cells/L. During the rest of the study, nucleated cell count values ranged from 1.6 to 38.4 x 10⁹ cells/L with a median of 4.7 x 10⁹ cells/L. Values of nucleated cell count peaked at 24 h and were significantly higher than baseline values at all time points, except at 96 h ($p = 0.138$) (Figure 7).

Baseline values for percentage of neutrophils ranged from 1 to 64% (median 23%). Throughout the study, values ranged from 21 to 78% with a median of 56%. There was no significant difference when compared baseline values ($p > 0.05$) to the rest of the sampling times (Figure 8).

Serum amyloid A concentrations in synovial fluid were below the reported lower limit of quantification of the assay (LOQ; 0.2 mg/L)^{94,164} at baseline; and remained <LOQ during the rest of the study (Figure 9). Systemic SAA baseline concentrations ranged from <LOQ to 0.6 mg/L with a median concentration of <LOQ. Throughout the study, systemic SAA values were \leq LOQ with a median of <LOQ (Figure 10). Due to a small number of samples with values above the LOQ, SAA results could not be compared statistically.

4.5 Discussion

The results of this study showed that systemic and synovial concentrations of SAA were not altered by repeated through-and-through joint lavage, whereas total protein concentration and nucleated cell count values were altered. Additionally, increased concentrations of SAA in synovial fluid have been described during septic arthritis.^{189,190} Therefore, synovial SAA concentrations may be worthy of measuring to monitor treatment effect of repeated through-and-through joint lavage in horses with septic arthritis.

To the authors' knowledge this is the first report that has evaluated the effect of repeated through-and-through joint lavage on synovial inflammatory markers and direct comparison with other studies is not possible. However, reports after a single through-and-through joint lavage have shown a significant increase in total protein and nucleated cell count values in synovial fluid (≤ 53 g/L and $\leq 38 \times 10^9$ cells/L, respectively).^{51,82,83} In these studies, these inflammatory markers reached values that have been associated with synovial sepsis 24 h after the procedure, similar to the results found in our study.

Previous studies have reported percentages of neutrophils values to be significantly increased 24 h after a single through-and-through joint lavage to values higher than the reference value for septic arthritis.^{80,82,83} We hypothesized that percentage of neutrophils will be significantly different from baseline values and the results of our study fail to prove this part of our hypothesis. However, the high variability of values obtained at the beginning of our study, possibly due to sample contamination with blood as shown by our statistical analysis, could be responsible for this observation.

The majority of our samples had evidence of blood contamination after the first lavage and occurred throughout the rest of the study. This alteration in the color of synovial fluid after a single through-and-through joint lavage has been reported in other studies,^{80,82,83} supporting our results.

In a previous clinical report, an average of three through-and-through joint lavage procedures were needed to resolve sepsis⁵⁷ and our study was design to simulate this clinical scenario. Although several practitioners prefer placing needles in the palmar/plantar aspect of the joint in selected cases, the placement of two needles and the use of three liters of LRS in our study was enough to produce a moderate inflammatory response characterized by increased synovial total protein concentration and nucleated cell count to values associated with sepsis 24 h after the lavage. The effect of using more liters of LRS is unknown and warrants further investigation. On the other hand, the authors believe that placing more needles would likely have not modified the clinical significance of our results.

A second group of tarsocrural joints receiving only repeated arthrocentesis and serving as controls was not implemented and maybe considered a limitation of the study. Repeated arthrocentesis has been evaluated in other experimental studies resulting in no changes on systemic and synovial SAA.^{94,189,208} However, the common synovial markers were reported to be

constantly altered after repeated arthrocentesis in previous studies.^{94,189,203,208} Inclusion of a control group sustaining repeated arthrocentesis was therefore considered unnecessary.

Few studies have focused on assessing and monitoring treatment for septic arthritis. Persistent synovial infection during treatment demonstrated by positive bacterial culture reduces overall prognosis² and it has been suggested that elevated nucleated cell count ($>1 \times 10^9$ cells/L) and percentage of neutrophils ($>30\%$) at 4 to 6 days after initiating treatment inferred a poor prognosis for athletic function.¹² Also a total protein concentration of 50 to 55 g/L obtained after arthroscopy was recently associated with reduce likelihood of survival to hospital discharge.¹⁹ Similar changes in total protein, nucleated cell count and percentage of neutrophils have been reported in healthy joints after repeated intra-articular administration of amikacin,⁹⁴ arthroscopic lavage,²⁰⁸ and in the present study, after repeated through-and-through joint lavage. Therefore, there is a need for a marker that could assess promptly failure of treatment. This will help clinicians on making accurate and early decisions to adjust therapy accordingly as well as obtaining a precise prognosis.

Serum amyloid A concentrations in healthy joints have been reported to be below the LOQ^{94,189,208} similar to our results. During septic arthritis these concentrations regularly increase to high values and decrease when clinical improvement is noticed.^{189,190} In our study, and as hypothesized, we proved that through-and-through joint lavage has no effect in systemic and synovial SAA as all values were $<LOQ$. Further evaluation of SAA concentrations in synovial fluid as a marker to assess resolution of sepsis while treatment for septic arthritis is installed, is warranted.

Based on the results of this study, we believe that SAA may be a valuable marker to be used for monitoring sepsis during aggressive treatment with repeated through-and-through joint lavage. Additional prospective experimental studies evaluating synovial SAA during treatment of clinical cases of septic arthritis are needed before recommending its use.

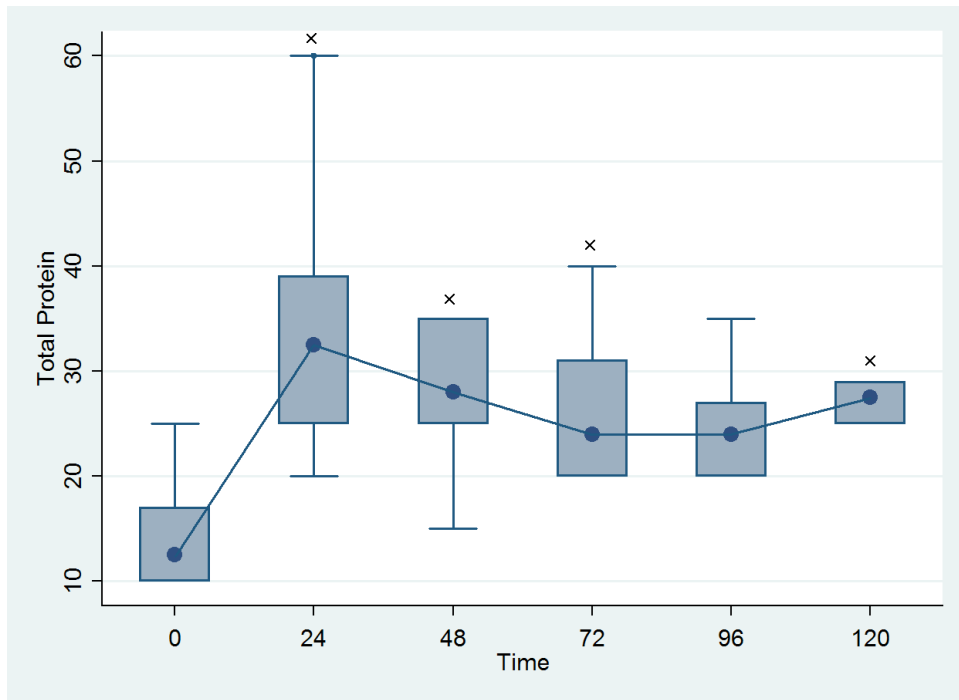


Figure 6. Box plot of median (circles) total protein concentrations (g/L) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. *Significant difference ($p < 0.05$) from baseline.

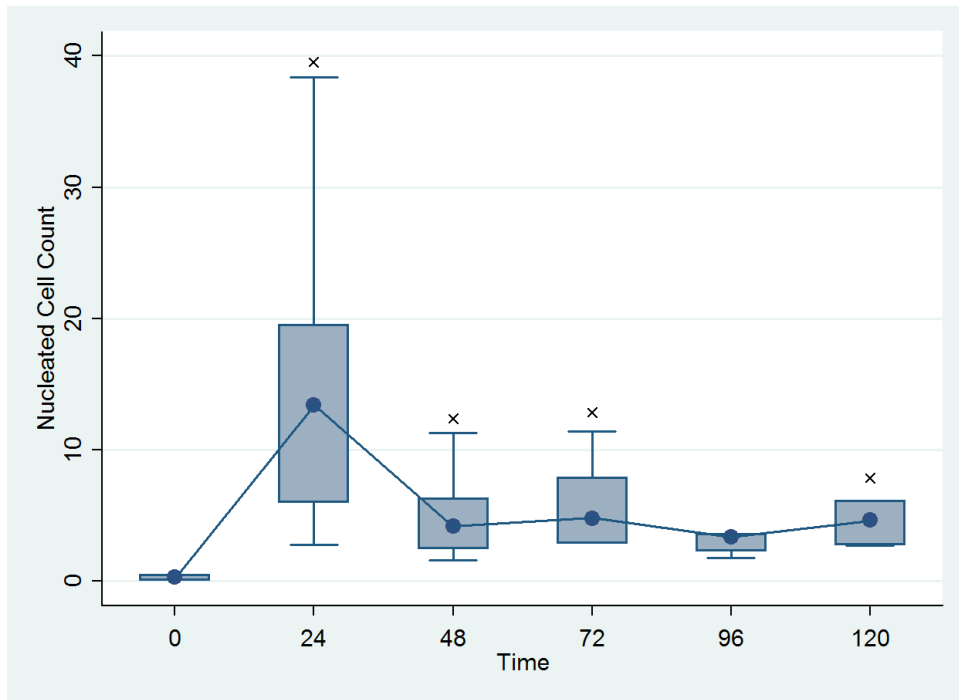


Figure 7. Box plot of median (circles) nucleated cell counts ($\times 10^9$ cells/L) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ^xSignificant difference ($p < 0.05$) from baseline.

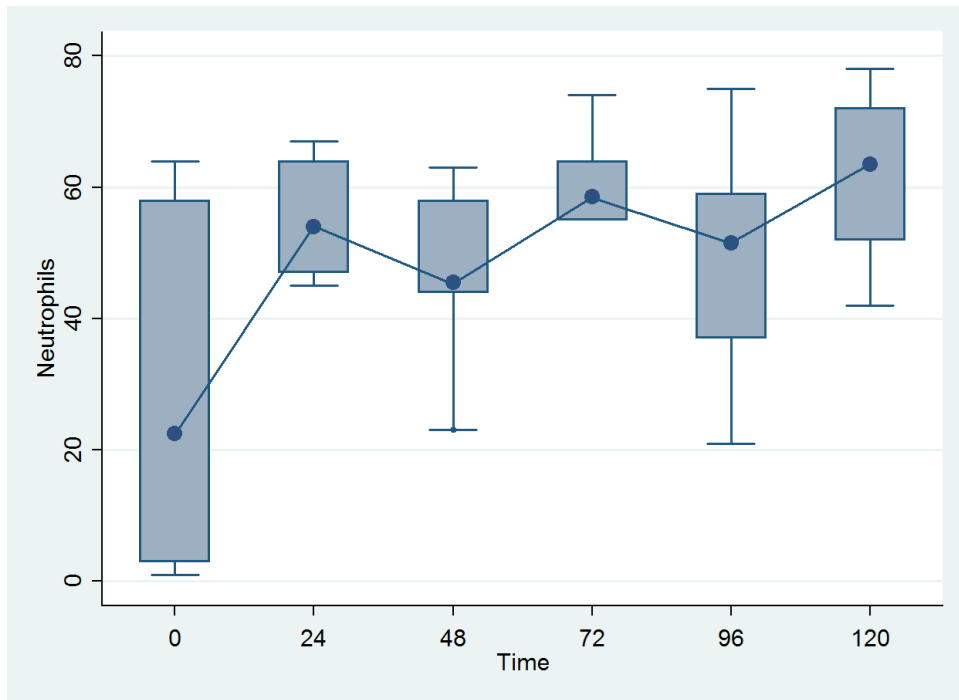


Figure 8. Box plot of median (circles) neutrophils (%) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. *Significant difference ($p < 0.05$) from baseline.

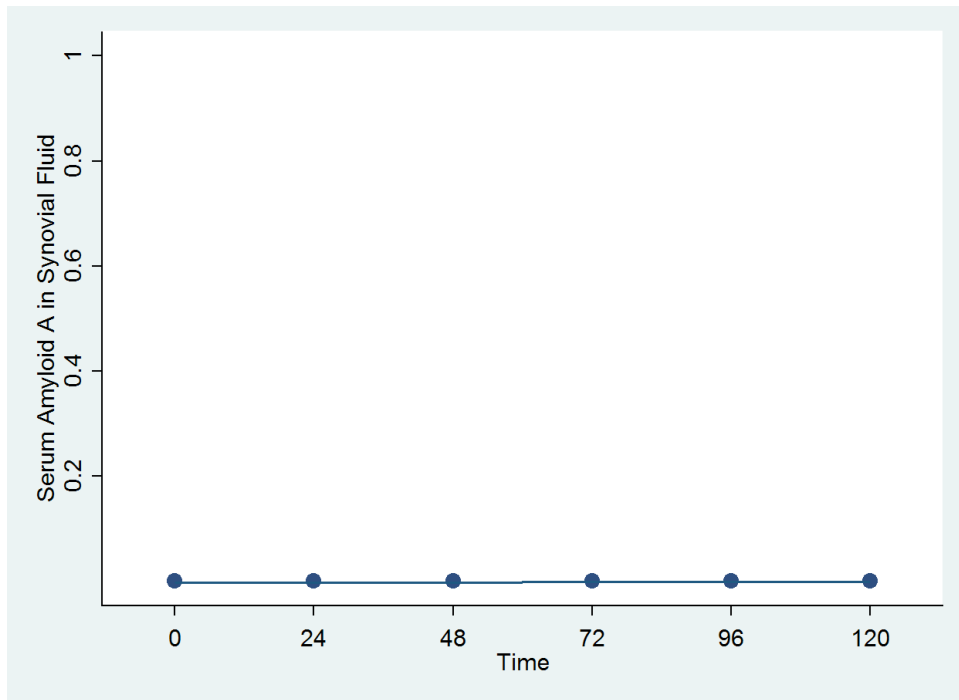


Figure 9. Box plot of median (circles) serum amyloid A concentrations (mg/L) in synovial fluid after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ^xSignificant difference ($p < 0.05$) from baseline.

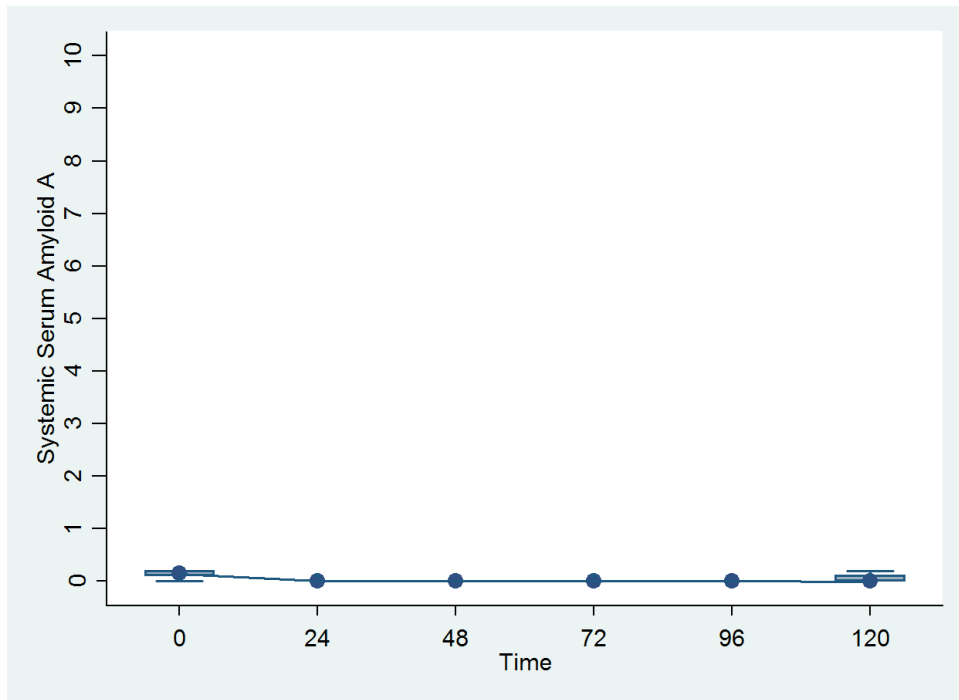


Figure 10. Box plot of median (circles) systemic serum amyloid A concentrations (mg/L) after repeated through-and-through joint lavage in 6 healthy horses. Bottom and top of box corresponds to 25th and 75th percentiles, respectively. Whiskers correspond to maximum and minimum values. ^xSignificant difference ($p<0.05$) from baseline.

CHAPTER 5: GENERAL DISCUSSION

The effects of arthroscopic lavage and repeated through-and-through joint lavage on systemic and synovial SAA concentration, total protein concentration, nucleated cell count and percentage of neutrophils were successfully evaluated and discussed through this thesis. Although these procedures affected the common synovial inflammatory markers, SAA concentrations were not altered. The concentrations of SAA detected in our studies were low. Most of systemic and synovial SAA concentrations were <LOQ of the assay and remained low throughout the studies. In previous research, SAA concentrations were below the LOQ in healthy joints and increased in 100 to 1000-fold in septic synovial fluid.^{189,190} Also, repeated arthrocentesis and repeated intra-articular injections of amikacin have been tested before, resulting in no increase on systemic or synovial SAA concentrations.^{94,189} The fact that SAA is not affected by treatment for septic arthritis, but is increased during infection, might be of advantage when using SAA as a marker for resolution of sepsis while treatment is applied. Clinical studies evaluating SAA concentrations in synovial fluid are still needed before final recommendation of its use is given.

Arthroscopic lavage and repeated through-and-through joint lavage had a major effect on the common synovial inflammatory markers. In other reports, these markers were also affected by repeated arthrocentesis and repeated intra-articular administration of amikacin.^{94,189} Therefore, their sole use to monitor treatment success or failure is not recommended. A recent retrospective report of horses treated for septic arthritis argues this idea by claiming that horses with normal synovial fluid analysis at 4 to 6 days post-lavage had a better prognosis than horses with altered synovial inflammatory markers, and that the effect of treatment procedures on the common synovial markers is unfounded.¹² This thesis along with two previous reports in normal horses^{78,79} have shown that the synovial inflammatory markers changes persist at 5 days post-lavage and even at 28 days after lavage. Further trials in horses with experimentally induced septic arthritis should be performed to evaluate the effect of treatment under these circumstances.

After general anesthesia, SAA had a mild increase in synovial fluid and a moderate increase in its systemic concentrations on two control horses and in one horse that received arthroscopic lavage. As discussed in the thesis, the effect of anesthesia on SAA is still unknown and an increase in systemic concentrations of SAA may have a significant effect on synovial

SAA concentrations. This might be of concern when assessing synovial fluid SAA concentrations in horses that have an ongoing systemic disease. Special caution should be taken when evaluating joint sepsis on septic foals with increased values of SAA in blood. Further studies evaluating the effect of systemic inflammation on synovial concentrations of SAA need to be performed before recommending SAA as a specific marker for septic synovitis.

Synovial SAA concentrations could serve a specific (and still unknown) function during septic synovitis. This is supported by an in-vitro study where SAA was found to bind to the outer membrane protein A of Gram-negative bacteria.¹⁴⁵ Another study also showed that SAA had LPS-binding capacity.¹⁴⁶ These reported biological properties of SAA and the fact that SAA is not affected by procedures that induce non-septic inflammation, suggests that SAA may have a specific role related to bacterial infection and elimination, which warrants further investigation.

The lack of changes in the concentrations of SAA in synovial fluid after high volume lavage by the two exposed procedures might be due to absence of a systemic stimulus to induce hepatic and maybe local production of SAA. After reviewing the current literature, there is only one study that mentions local synthesis of SAA within the joint and without a systemic response. This was reported in horses with osteoarthritis on which more synovial fluid samples had SAA concentrations above the LOQ of the assay than serum samples.¹⁸⁹ The values found above the LOQ were still low values of SAA. This suggests that although local synthesis of SAA within the joint is present, this may not represent a great quantity of the total SAA measured and that the majority of SAA in the joint might originate from hepatic synthesis. Further studies in this aspect are still needed.

The two studies within this thesis show that SAA is not affected by arthroscopic lavage and repeated through-and-through joint lavage. The known increase in synovial SAA concentrations during septic synovitis and the lack of change in synovial SAA concentrations reported here; suggests that quantification of synovial SAA over the common synovial inflammatory markers when evaluating a synovial cavity for sepsis that has undergone these procedures, could help in the monitoring of treatment. Further studies quantifying SAA through treatment of naturally-occurring or experimentally-induced septic synovitis are warranted.

CHAPTER 6: CONCLUSIONS AND FUTURE STUDIES

Based on the results of the presented studies we can conclude that:

- Arthroscopic lavage of the middle carpal joint in healthy horses under general anesthesia:
 - Significantly increased total protein concentrations in synovial fluid with some values being within the reference values for synovial sepsis.
 - Significantly increased nucleated cell counts in synovial fluid to values higher than normal but below the reference range for septic synovitis.
 - Significantly increased the percentage of neutrophils in synovial fluid throughout the study period, with values consistent with septic synovitis at 24 h and 48 h.
 - Did not have an effect on SAA concentrations in synovial fluid.
 - Significantly increased SAA concentrations in blood at 24 h, likely as a result from anesthesia or procedures related to anesthesia.
- Repeated through-and-through lavage of the tarsocrural joint in healthy horses under general anesthesia:
 - Significantly increased total protein concentrations in synovial fluid, with values above the reference range for septic synovitis at 24 h and 72 h.
 - Significantly increased nucleated cell counts in synovial fluid to values above the reference range for septic synovitis at 24 h.
 - Did not have an effect on percentages of neutrophils in synovial fluid. However, some percentages approximate the reference range for septic synovitis.
 - Did not have an effect on systemic and synovial fluid SAA concentrations.
- Repeated arthrocentesis of the middle carpal joint in healthy horses:
 - Did not significantly affect total protein concentrations in synovial fluid. However, some values were above the reference values for septic synovitis at 96 h.
 - Significantly increased nucleated cell counts in synovial fluid. However, values were below the reference range for septic synovitis.
 - Significantly increased the percentage of neutrophils in synovial fluid only at 24 h after the first arthrocentesis.
 - Did not have an effect on systemic and synovial fluid SAA concentrations.

The limitations of the presented studies include:

- The effects of lavage procedures in healthy joints. The effect of these procedures on the studied inflammatory markers during septic synovitis may vary and differ from our results.
- In a clinical setting, the studied lavage procedures are commonly used in combination with administration of local and systemic antimicrobials as well as systemic anti-inflammatories. Therefore, the effect of these procedures combined might differ from the results exposed in this thesis.
- The assay used for quantification of SAA on the presented studies behaved in a reliable manner and has been validated for use in equine serum. However, formal validation of the assay for its use in equine synovial fluid is needed before recommending its clinical use.

Future studies considered necessary by the author of this thesis include:

The current literature available suggests that synovial SAA increases during septic synovitis and through this thesis we have demonstrated that is not affected by the studied lavage procedures and therefore, SAA may serve as a good marker for diagnosis as well as monitoring treatment of septic synovitis. Although a recent study evaluating a semi-quantitative colorimetric SAA test has shown good specificity and sensitivity when differentiating between non-septic and septic arthritis, the authors of the study claim that more clinical cases are needed to validate their results.¹⁹⁰ Therefore, new studies should focus on validation of SAA as a marker for septic arthritis during clinical or experimentally induced disease. These studies should include analysis of synovial fluid SAA during acute and sub-acute stages. Determination of positive and negative predictive values of serum and synovial fluid SAA concentrations are necessary before recommending SAA as a marker for septic synovitis.

The current assays validated for equine use include a human turbidometric immunoassay¹⁶⁴ and a human latex agglutination immunoassay.¹⁹⁵ These two assays are validated for use in equine serum and the former has been widely used in studies on equine synovial fluid. However, a formal validation of the assay for use in synovial fluid should be performed to obtain intra- and inter-assay variability values. Development of an assay using

equine anti-SAA antibodies might bring advantages over human SAA assays and should be attempted.¹⁹⁶

The reported values of SAA in synovial fluid from healthy joints range from <0.2 to 0.7 mg/L.⁹⁴ Only one report had values of synovial SAA higher than 0.7 mg/L with values of ≤ 4 mg/L found in healthy joints.¹⁹¹ New studies should focus on obtaining reference values of SAA in synovial fluid of healthy horses. These should also include evaluation of SAA as a marker of involvement of bone or tendon in the septic processes and if SAA could serve as a prognostic indicator for survival and return to athletic performance.

Quantification of the different SAA isoforms remains a challenge. Isoforms have slight molecular differences that need to be described before assays can be developed. These differences might be very small and the assays will need to be highly specific to differentiate between them. The value of developing isoform-specific assays will be dependent on the increase of the different isoforms during pathologic processes. This increase may not be to concentrations high enough for the assay to detect them or be significant. Studies regarding SAA isoform-specific increases during pathologic processes could be advantageous in elucidating the varied functions of SAA and further direct therapy for specific processes.

Future studies on SAA in blood and synovial fluid as well as in other bodily fluids or tissues are vast. Elucidation of the biological functions of SAA as well as the use of SAA as a marker during different diseases and their stages are needed and warrant several years of intense research.

CHAPTER 7: MASTER REFERENCE LIST

1. Anderson DM, in Dorland's Medical Dictionary (32nd Edition), Vol 2015. Philadelphia, PA, Elsevier, 2014, p 150.
2. Taylor AH, Mair TS, Smith LJ, et al: Bacterial culture of septic synovial structures of horses: does a positive bacterial culture influence prognosis? *Equine Vet J* 42:213-218, 2010.
3. McIlwraith CW: Treatment of Infectious Arthritis. *Vet Clin N Am-Large* 5:363-379, 1983.
4. Wright IM, Smith MR, Humphrey DJ, et al: Endoscopic surgery in the treatment of contaminated and infected synovial cavities. *Equine Vet J* 35:613-619, 2003.
5. Martens RJ, Auer JA, Carter GK: Equine Pediatrics - Septic Arthritis and Osteomyelitis. *Journal of the American Veterinary Medical Association* 188:582-585, 1986.
6. Morton AJ: Diagnosis and treatment of septic arthritis. *Vet Clin N Am-Equine* 21:627, 2005.
7. Borg H, Carmalt JL: Postoperative septic arthritis after elective equine arthroscopy without antimicrobial prophylaxis. *Vet Surg* 42:262-266, 2013.
8. Lapointe JM, Laverty S Fau - Lavoie JP, Lavoie JP: Septic arthritis in 15 standardbred racehorses after intra-articular injection. *Equine Vet J*:430-434, 1992.
9. Bertone AL: Update on infectious arthritis in horses. *Equine Vet Ed* 11:143-152, 1999.
10. Frisbie D: Synovial joint biology and pathobiology, in Auer JA, J. S (eds): *Equine Surgery* (ed Third), Vol. St. Louis Missouri, Elsevier Inc., 2006, pp 1036-1055.
11. Schneider RK, Bramlage LR, Moore RM, et al: A retrospective study of 192 horses affected with septic arthritis/tenosynovitis. *Equine Vet J* 24:436-442, 1992.
12. Walmsley EA, Anderson GA, Muurlink MA, et al: Retrospective investigation of prognostic indicators for adult horses with infection of a synovial structure. *Aust Vet J* 89:226-231, 2011.
13. Raisis AL, Hodgson JL, Hodgson DR: Equine neonatal septicaemia: 24 cases. *Aust Vet J* 73:137-140, 1996.
14. Smith LJ, Marr CM, Payne RJ, et al: What is the likelihood that Thoroughbred foals treated for septic arthritis will race? *Equine Vet J* 36:452-456, 2004.

15. Frees KE, Lillich JD, Gaughan EM, et al: Tenoscopic-assisted treatment of open digital flexor tendon sheath injuries in horses: 20 cases (1992-2001). *J Am Vet Med Assoc* 220:1823-1827, 2002.
16. Fraser BS, Bladon BM: Tenoscopic surgery for treatment of lacerations of the digital flexor tendon sheath. *Equine Vet J* 36:528-531, 2004.
17. Honnas CM, Schumacher J, Cohen ND, et al: Septic tenosynovitis in horses: 25 cases (1983-1989). *J Am Vet Med Assoc* 199:1616-1622, 1991.
18. Wereszka MM, White NA, 2nd, Furr MO: Factors associated with outcome following treatment of horses with septic tenosynovitis: 51 cases (1986-2003). *J Am Vet Med Assoc* 230:1195-1200, 2007.
19. Milner PI, Bardell DA, Warner L, et al: Factors associated with survival to hospital discharge following endoscopic treatment for synovial sepsis in 214 horses. *Equine Vet J* 46:701-705, 2014.
20. Mostafa MB, Abu-Seida AM, El-Glil AIA: Septic tarsitis in horses: clinical, radiological, ultrasonographic, arthroscopic and bacteriological findings. *Res Opin Anim Vet Sci* 4:30-34, 2014.
21. Kidd JA, Barr AR, Tarlton JF: Use of matrix metalloproteinases 2 and 9 and white blood cell counts in monitoring the treatment and predicting the survival of horses with septic arthritis. *Vet Rec* 161:329-334, 2007.
22. Gibson KT, McIlwraith CW, Turner AS, et al: Open joint injuries in horses: 58 cases (1980-1986). *J Am Vet Med Assoc* 194:398-404, 1989.
23. Ross MW, Orsini JA, Richardson DW, et al: Closed suction drainage in the treatment of infectious arthritis of the equine tarsocrural joint. *Vet Surg* 20:21-29, 1991.
24. Madison JB, Sommer M, Spencer PA: Relations among synovial membrane histopathologic findings, synovial fluid cytologic findings, and bacterial culture results in horses with suspected infectious arthritis: 64 cases (1979-1987). *J Am Vet Med Assoc* 198:1655-1661, 1991.
25. Orsini JA: Strategies for treatment of bone and joint infections in large animals. *J Am Vet Med Assoc* 185:1190-1193, 1984.

26. Tulamo RM, Bramlage LR, Gabel AA: Sequential Clinical and Synovial-Fluid Changes Associated with Acute Infectious Arthritis in the Horse. *Equine Vet J* 21:325-331, 1989.
27. Moore RM, Schneider RK, Kowalski J, et al: Antimicrobial Susceptibility of Bacterial Isolates from 233 Horses with Musculoskeletal Infection during 1979-1989. *Equine Vet J* 24:450-456, 1992.
28. Bohlin AM, Kristoffersen M, Toft N: Infectious arthritis following intra-articular injection in horses not receiving prophylactic antibiotics: A retrospective cohort study of 2833 medical records, Proceedings of the Annual Meeting of the American Association of Equine Practitioners, Salt Lake City, Utah, 2014
29. Reginato AJ, Ferreiro JL, O'Connor CR, et al: Clinical and pathologic studies of twenty-six patients with penetrating foreign body injury to the joints, bursae, and tendon sheaths. *Arthritis Rheum* 33:1753-1762, 1990.
30. Steel CM, Hunt AR, Adams PL, et al: Factors associated with prognosis for survival and athletic use in foals with septic arthritis: 93 cases (1987-1994). *J Am Vet Med Assoc* 215:973-977, 1999.
31. Stoneham SJ: Septic arthritis in the foal: Practical considerations on diagnosis and treatment. *Equine Vet Edu* 9:25-29, 1997.
32. Tizard RI: How inflammation is triggered, in *Veterinary Immunology: An Introduction* (ed Seven Edition), Vol. Philadelphia, Saunders, Elsevier, 2004, pp 10-23.
33. Hardy J, Bertone AL, Malemud CJ: Effect of synovial membrane infection in vitro on equine synoviocytes and chondrocytes. *Am J Vet Res* 59:293-299, 1998.
34. Spiers S, May SA, Harrison LJ, et al: Proteolytic enzymes in equine joints with infectious arthritis. *Equine Vet J* 26:48-50, 1994.
35. Palmer JL, Bertone AL: Joint structure, biochemistry and biochemical disequilibrium in synovitis and equine joint disease. *Equine Vet J* 26:263-277, 1994.
36. Curtiss PH, Jr.: Changes Produced in the Synovial Membrane and Synovial Fluid by Disease. *J Bone Joint Surg Am* 46:873-888, 1964.
37. Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448-454, 1999.

38. Steel DM, Whitehead AS: The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today* 15:81-88, 1994.
39. Yamashita K, Fujinaga T, Okumura M, et al: Serum C-reactive protein (CRP) in horses: the effect of aging, sex, delivery and inflammations on its concentration. *J Vet Med Sci* 53:1019-1024, 1991.
40. Jacobsen S, Andersen PH: The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Vet Edu* 19:38-46, 2007.
41. Wright IM: Arthroscopic surgery in the management of contamination and infection of joints, tendon sheaths, and bursae. *Clin Tech Equine P* 1:234-244, 2002.
42. Firth EC: Current Concepts of Infectious Polyarthrititis in Foals. *Equine Vet J* 15:5-9, 1983.
43. Honnas CM, Welch RD, Ford TS, et al: Septic arthritis of the distal interphalangeal joint in 12 horses. *Vet Surg* 21:261-268, 1992.
44. Ross MW, Dyson SJ: Chapter 7: Movement, in: *Diagnosis and Management of Lameness in the Horse* (ed 2nd), Vol. St. Louis, Missouri, Elsevier Inc., 2011, pp 64-80.
45. Ghanem E, Parvizi J Fau - Clohisy J, Clohisy J Fau - Burnett S, et al: Perioperative antibiotics should not be withheld in proven cases of periprosthetic infection. *Clin Orthop* 461:44-47, 2007.
46. DeGara PF: Studies on the Bactericidal Properties of the Synovial Fluid. *J Clin Invest* 22:131-136, 1943.
47. Gruber BF, Miller BS, Onnen J, et al: Antibacterial properties of synovial fluid in the knee. *J Knee Surg* 21:180-185, 2008.
48. Dumoulin M, Pille F, van den Abeele AM, et al: Use of blood culture medium enrichment for synovial fluid culture in horses: a comparison of different culture methods. *Equine Vet J* 42:541-546, 2010.
49. Lavoie JP, Couture L, Higgins R, et al: Aerobic bacterial isolates in horses in a university hospital, 1986-1988. *Can Vet J* 32:292-294, 1991.
50. Weston VC, Jones AC, Bradbury N, et al: Clinical features and outcome of septic arthritis in a single UK Health District 1982-1991. *Ann Rheum Dis* 58:214-219, 1999.

51. Bertone AL, McIlwraith CW, Jones RL, et al: Comparison of various treatments for experimentally induced equine infectious arthritis. *Am J Vet Res* 48:519-529, 1987.
52. Montgomery RD, Long IR, Jr., Milton JL, et al: Comparison of aerobic culturette, synovial membrane biopsy, and blood culture medium in detection of canine bacterial arthritis. *Vet Surg* 18:300-303, 1989.
53. Steel CM: Equine synovial fluid analysis. *Vet Clin N Am-Equine* 24:437, 2008.
54. Van Pelt RW: Interpretation of synovial fluid findings in the horse. *J Am Vet Med Assoc* 165:91-95, 1974.
55. McIlwraith CW: Synovial fluid analysis in the diagnosis of equine joint disease. *Equine P* 44-48, 1980.
56. Stutz G, Kuster MS, Kleinstuck F, et al: Arthroscopic management of septic arthritis: stages of infection and results. *Knee Surg Sports Traumatol Arthrosc* 8:270-274, 2000.
57. Meijer MC, van Weeren PR, Rijkenhuizen AB: Clinical experiences of treating septic arthritis in the equine by repeated joint lavage: a series of 39 cases. *J Vet Med A Physiol Pathol Clin Med* 47:351-365, 2000.
58. Schneider RK, Bramlage LR: Recommendations for the clinical management of septic arthritis in horses, *Proceedings of the American Association of Equine Practitioners*, 1991.
59. Schneider RK, Bramlage LR, Mecklenburg LM, et al: Open drainage, intra-articular and systemic antibiotics in the treatment of septic arthritis/tenosynovitis in horses. *Equine Vet J* 24:443-449, 1992.
60. Martens RJ, Auer JA: Hematogenous septic arthritis and osteomyelitis in the foal, *Proceedings of the American Association of Equine Practitioners*, 1980.
61. Bertone AL, Davis DM, Cox HU, et al: Arthrotomy versus arthroscopy and partial synovectomy for treatment of experimentally induced infectious arthritis in horses. *Am J Vet Res* 53:585-591, 1992.
62. Santschi EM, Adams SB, Fessler JF, et al: Treatment of bacterial tarsal tenosynovitis and osteitis of the sustentaculum tali of the calcaneus in five horses. *Equine Vet J* 29:244-247, 1997.

63. Chan CC, Murphy H, Munroe GA: Treatment of chronic digital septic tenosynovitis in 12 horses by modified open annular ligament desmotomy and passive open drainage. *Vet Rec* 147:388-393, 2000.
64. Anderson DE, Allen D, St-Jean G, et al: Use of a multifenestrated indwelling lavage system for treatment of septic digital tenosynovitis in cattle. *Aust Vet J* 75:796-799, 1997.
65. Honnas CM, Schumacher J, Kuesis BS: Ankylosis of the distal interphalangeal joint in a horse after septic arthritis and septic navicular bursitis. *J Am Vet Med Assoc* 200:964-968, 1992.
66. Booth TM, Abbot J, Clements A, et al: Treatment of septic common digital extensor tenosynovitis by complete resection in seven horses. *Vet Surg* 33:107-111, 2004.
67. Welch RD, Watkins JP, DeBowes RM, et al: Effects of intra-articular administration of dimethylsulfoxide on chemically induced synovitis in immature horses. *Am J Vet Res* 52:934-939, 1991.
68. Owens JG, Kamerling SG, Stanton SR, et al: Effects of pretreatment with ketoprofen and phenylbutazone on experimentally induced synovitis in horses. *Am J Vet Res* 57:866-874, 1996.
69. Raekallio M, Taylor PM, Bennett RC: Preliminary investigations of pain and analgesia assessment in horses administered phenylbutazone or placebo after arthroscopic surgery. *Vet Surg* 26:150-155, 1997.
70. Snow DH, Douglas TA, Thompson H, et al: Phenylbutazone toxicity in ponies. *Vet Rec* 106:68, 1980.
71. MacKay RJ, French TW, Nguyen HT, et al: Effects of large doses of phenylbutazone administration to horses. *Am J Vet Res* 44:774-780, 1983.
72. Kvaternick V, Pollmeier M, Fischer J, et al: Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. *J Vet Pharmacol Ther* 30:208-217, 2007.
73. Orsini JA, Ryan WG, Carithers DS, et al: Evaluation of oral administration of firocoxib for the management of musculoskeletal pain and lameness associated with osteoarthritis in horses. *Am J Vet Res* 73:664-671, 2012.

74. Doucet MY, Bertone AL, Hendrickson D, et al: Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc* 232:91-97, 2008.
75. Brusie RW, Sullins KE, White NA, 2nd, et al: Evaluation of sodium hyaluronate therapy in induced septic arthritis in the horse. *Equine Vet J Suppl*:18-23, 1992.
76. Gustafson SB, McIlwraith CW, Jones RL: Comparison of the effect of polysulfated glycosaminoglycan, corticosteroids, and sodium hyaluronate in the potentiation of a subinfective dose of *Staphylococcus aureus* in the midcarpal joint of horses. *Am J Vet Res* 50:2014-2017, 1989.
77. Ayral X: Arthroscopy and joint lavage. *Best Pract Res Clin Rheumatol* 19:401-415, 2005.
78. Rossetti RB, Massoco CO, Alves Penna AC, et al: An experimental study to compare inflammatory response due to liquid or gas joint distension in horses submitted to arthroscopy. *Acta Cir Bras* 27:848-854, 2012.
79. Jones DL, Barber SM, Doige CE: Synovial fluid and clinical changes after arthroscopic partial synovectomy of the equine middle carpal joint. *Vet Surg* 22:524-530, 1993.
80. Bertone AL, McIlwraith CW, Powers BE, et al: Effect of Four Antimicrobial lavage solutions on the tarsocrural joint of horses. *Vet Surg* 15:305-315, 1986.
81. Bertone AL, McIlwraith CW, Jones RL, et al: Povidone-iodine lavage treatment of experimentally induced equine infectious arthritis. *Am J Vet Res* 48:712-715, 1987.
82. Wilson DG, Cooley AJ, MacWilliams PS, et al: Effects of 0.05% chlorhexidine lavage on the tarsocrural joints of horses. *Vet Surg* 23:442-447, 1994.
83. Klohnen A, Wilson DG, Hendrickson DA, et al: Effects of potentiated chlorhexidine on bacteria and tarsocrural joints in ponies. *Am J Vet Res* 57:756-761, 1996.
84. Schneider RK: Common bacteria encountered in septic arthritis, *Proceedings of the Annual Convention of the American Association of Equine Practitioners*, 1998.
85. Seguin JC, Walker RD, Caron JP, et al: Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *J Clin Microbiol* 37:1459-1463, 1999.
86. Trostle SS, Peavey CL, King DS, et al: Treatment of methicillin-resistant *Staphylococcus epidermidis* infection following repair of an ulnar fracture and humero-radial joint luxation in a horse. *J Am Vet Med Assoc* 218:554-559, 527, 2001.

87. Rubio-Martinez LM, Elmas CR, Black B, et al: Clinical use of antimicrobial regional limb perfusion in horses: 174 cases (1999-2009). *J Am Vet Med Assoc* 241:1650-1658, 2012.
88. Mills ML, Rush BR, St Jean G, et al: Determination of synovial fluid and serum concentrations, and morphologic effects of intraarticular ceftiofur sodium in horses. *Vet Surg* 29:398-406, 2000.
89. Lloyd KC, Stover SM, Pascoe JR, et al: Synovial fluid pH, cytologic characteristics, and gentamicin concentration after intra-articular administration of the drug in an experimental model of infectious arthritis in horses. *Am J Vet Res* 51:1363-1369, 1990.
90. Taintor J, Schumacher J, DeGraves F: Comparison of amikacin concentrations in normal and inflamed joints of horses following intra-articular administration. *Equine Vet J* 38:189-191, 2006.
91. Clark C, Greenwood S, Dowling PM: A review of bacterial isolates of equine origin (1998–2003) and implications for antimicrobial therapy. *J Vet Pharmacol Ther* 27:385-387, 2004.
92. Sedrich SA, Moore RM, Barker SA: Pharmacokinetics of single dose intra-articular administration of amikacin in the radiocarpal joints of normal horses, *Proceedings of the Surgery Meeting of the American College of Veterinary Surgeons*, Illinois, 1994.
93. Bolt DM, Ishihara A, Weisbrode SE, et al: Effects of triamcinolone acetone, sodium hyaluronate, amikacin sulfate, and mepivacaine hydrochloride, alone and in combination, on morphology and matrix composition of lipopolysaccharide-challenged and unchallenged equine articular cartilage explants. *Am J Vet Res* 69:861-867, 2008.
94. Sanchez Teran AF, Rubio-Martinez LM, Villarino NF, et al: Effects of repeated intra-articular administration of amikacin on serum amyloid A, total protein and nucleated cell count in synovial fluid from healthy horses. *Equine Vet J* 44 Suppl 43:12-16, 2012.
95. Lescun TB, Adams SB, Wu CC, et al: Effects of continuous intra-articular infusion of gentamicin on synovial membrane and articular cartilage in the tarsocrural joint of horses. *Am J Vet Res* 63:683-687, 2002.

96. Lescun TB, Adams SB, Wu CC, et al: Continuous infusion of gentamicin into the tarsocrural joint of horses. *Am J Vet Res* 61:407-412, 2000.
97. Stewart AA, Goodrich LR, Byron CR, et al: Antimicrobial delivery by intrasynovial catheterisation with systemic administration for equine synovial trauma and sepsis. *Aust Vet J* 88:115-123, 2010.
98. Cruz AM, Rubio-Martinez L, Dowling T: New Antimicrobials, Systemic Distribution, and Local Methods of Antimicrobial Delivery in Horses. *Vet Clin N Am-Equine* 22:297-322, 2006.
99. Tobias KM, Schneider RK, Besser TE: Use of antimicrobial-impregnated polymethyl methacrylate. *J Am Vet Med Assoc* 208:841-845, 1996.
100. Butson RJ, Schramme MC, Garlick MH, et al: Treatment of intrasynovial infection with gentamicin-impregnated polymethylmethacrylate beads. *Vet Rec* 138:460-464, 1996.
101. Whitehair KJ, Blevins WE, Fessler JF, et al: Regional perfusion of the equine carpus for antibiotic delivery. *Vet Surg* 21:279-285, 1992.
102. Whitehair KJ, Bowersock TL, Blevins WE, et al: Regional limb perfusion for antibiotic treatment of experimentally induced septic arthritis. *Vet Surg* 21:367-373, 1992.
103. Santschi EM, Adams SB, Murphey ED: How to perform equine intravenous digital perfusion, *Proceedings of the 44th Annual Convention of the American Association of Equine Practitioners*, 1998.
104. Pepys MB, Baltz ML: Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv Immunol* 34:141-212, 1983.
105. Husebekk A, Husby G, Sletten K, et al: Characterization of amyloid protein AA and its serum precursor SAA in the horse. *Scand J Immunol* 23:703-709, 1986.
106. Benditt EP, Eriksen N: Amyloid protein SAA is associated with high density lipoprotein from human serum. *Proceedings of the National Academy of Sciences of the United States of America* 74:4025-4028, 1977.
107. Berg LC, Thomsen PD, Andersen PH, et al: Serum amyloid A is expressed in histologically normal tissues from horses and cattle. *Vet Immunol Immunop* 144:155-159, 2011.

108. Morrow JF, Stearman RS, Peltzman CG, et al: Induction of hepatic synthesis of serum amyloid A protein and actin. *Proc Natl Acad Sci U S A* 78:4718-4722, 1981.
109. Ramadori G, Van Damme J, Rieder H, et al: Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. *Eur J Immunol* 18:1259-1264, 1988.
110. Bausserman LL, Saritelli AL, Van Zuiden P, et al: Degradation of serum amyloid A by isolated perfused rat liver. *J Biol Chem* 262:1583-1589, 1987.
111. Hoffman JS, Benditt EP: Plasma clearance kinetics of the amyloid-related high density lipoprotein apoprotein, serum amyloid protein (apoSAA), in the mouse. Evidence for rapid apoSAA clearance. *J Clin Invest* 71:926-934, 1983.
112. Sipe JD, Benson MD, Buxbaum JN, et al: Amyloid fibril protein nomenclature: 2010 recommendations from the nomenclature committee of the International Society of Amyloidosis. *Amyloid* 17:101-104, 2010.
113. Husebekk A, Skogen B, Husby G, et al: Transformation of amyloid precursor SAA to protein AA and incorporation in amyloid fibrils in vivo. *Scand J Immunol* 21:283-287, 1985.
114. Sletten K, Husebekk A, Husby G: The amino acid sequence of an amyloid fibril protein AA isolated from the horse. *Scand J Immunol* 26:79-84, 1987.
115. Dong Z, Wu T, Qin W, et al: Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Mol Med* 17:1357-1364, 2011.
116. Fyfe AI, Rothenberg LS, DeBeer FC, et al: Association between serum amyloid A proteins and coronary artery disease: evidence from two distinct arteriosclerotic processes. *Circulation* 96:2914-2919, 1997.
117. Uhlar CM, Whitehead AS: Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* 265:501-523, 1999.
118. Sletten K, Husebekk A, Husby G: The primary structure of equine serum amyloid A (SAA) protein. *Scand J Immunol* 30:117-122, 1989.
119. Nunokawa Y, Fujinaga T, Taira T, et al: Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci* 55:1011-1016, 1993.

120. Sletten K, Johnson KH, Westermarck P: The amino acid sequence of protein AA from a burro (*Equus asinus*). *Amyloid* 10:144-146, 2003.
121. Ma Z, Mizukoshi T, Khatlani TS, et al: Molecular cloning and sequencing of equine cDNA encoding serum amyloid A (SAA). *Vet Immunol Immunop* 77:321-327, 2000.
122. Uhlar CM, Burgess CJ, Sharp PM, et al: Evolution of the serum amyloid A (SAA) protein superfamily. *Genomics* 19:228-235, 1994.
123. Uhlar CM, Black IL, Shields DC, et al: Wallaby serum amyloid A protein: cDNA cloning, sequence and evolutionary analysis. *Scand J Immunol* 43:271-276, 1996.
124. Jensen LE, Hiney MP, Shields DC, et al: Acute phase proteins in salmonids: evolutionary analyses and acute phase response. *J Immunol* 158:384-392, 1997.
125. Santiago-Cardona PG, Berrios CA, Ramirez F, et al: Lipopolysaccharides induce intestinal serum amyloid A expression in the sea cucumber *Holothuria glaberrima*. *Dev Comp Immunol* 27:105-110, 2003.
126. Marhaug G, Hackett B, Dowton SB: Serum amyloid A gene expression in rabbit, mink and mouse. *Clin Exp Immunol* 107:425-434, 1997.
127. Jacobsen S, Niewold TA, Halling-Thomsen M, et al: Serum amyloid A isoforms in serum and synovial fluid in horses with lipopolysaccharide-induced arthritis. *Vet Immunol Immunop* 110:325-330, 2006.
128. Urieli-Shoval S, Cohen P, Eisenberg S, et al: Widespread expression of serum amyloid A in histologically normal human tissues. Predominant localization to the epithelium. *J Histochem Cytochem* 46:1377-1384, 1998.
129. Hulten C, Sletten K, Foyen Bruun C, et al: The acute phase serum amyloid A protein (SAA) in the horse: isolation and characterization of three isoforms. *Vet Immunol Immunop* 57:215-227, 1997.
130. Kluve-Beckerman B, Drumm ML, Benson MD: Nonexpression of the human serum amyloid A three (SAA3) gene. *DNA Cell Biol* 10:651-661, 1991.
131. Mukesh M, Bionaz M, Graugnard DE, et al: Adipose tissue depots of Holstein cows are immune responsive: inflammatory gene expression in vitro. *Domest Anim Endocrinol* 38:168-178, 2010.

132. McDonald TL, Larson MA, Mack DR, et al: Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. *Vet Immunol Immunop* 83:203-211, 2001.
133. Jacobsen S, Niewold TA, Kornalijslijper E, et al: Kinetics of local and systemic isoforms of serum amyloid A in bovine mastitic milk. *Vet Immunol Immunop* 104:21-31, 2005.
134. Pihl TH, Andersen PH, Kjelgaard-Hansen M, et al: Serum amyloid A and haptoglobin concentrations in serum and peritoneal fluid of healthy horses and horses with acute abdominal pain. *Vet Clin Pathol* 42:177-183, 2013.
135. Christoffersen M, Baagoe CD, Jacobsen S, et al: Evaluation of the systemic acute phase response and endometrial gene expression of serum amyloid A and pro- and anti-inflammatory cytokines in mares with experimentally induced endometritis. *Vet Immunol Immunop* 138:95-105, 2010.
136. Tolle M, Huang T, Schuchardt M, et al: High-density lipoprotein loses its anti-inflammatory capacity by accumulation of pro-inflammatory-serum amyloid A. *Cardiovasc Res* 94:154-162, 2012.
137. Kinkley SM, Bagshaw WL, Tam SP, et al: The path of murine serum amyloid A through peritoneal macrophages. *Amyloid* 13:123-134, 2006.
138. Yamada T, Wada A, Itoh Y, et al: Serum amyloid A1 alleles and plasma concentrations of serum amyloid A. *Amyloid* 6:199-204, 1999.
139. Kisilevsky R, Manley PN: Acute-phase serum amyloid A: perspectives on its physiological and pathological roles. *Amyloid* 19:5-14, 2012.
140. Linke RP, Bock V, Valet G, et al: Inhibition of the oxidative burst response of N-formyl peptide-stimulated neutrophils by serum amyloid-A protein. *Biochem Biophys Res Commun* 176:1100-1105, 1991.
141. Malle E, Bollmann A, Steinmetz A, et al: Serum amyloid A (SAA) protein enhances formation of cyclooxygenase metabolites of activated human monocytes. *FEBS Lett* 419:215-219, 1997.
142. Badolato R, Wang JM, Murphy WJ, et al: Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J Exp Med* 180:203-209, 1994.

143. Badolato R, Wang JM, Stornello SL, et al: Serum amyloid A is an activator of PMN antimicrobial functions: induction of degranulation, phagocytosis, and enhancement of anti-Candida activity. *J Leukoc Biol* 67:381-386, 2000.
144. Larson MA, Wei SH, Weber A, et al: Human serum amyloid A3 peptide enhances intestinal MUC3 expression and inhibits EPEC adherence. *Biochem Biophys Res Commun* 300:531-540, 2003.
145. Hari-Dass R, Shah C, Meyer DJ, et al: Serum amyloid A protein binds to outer membrane protein A of gram-negative bacteria. *J Biol Chem* 280:18562-18567, 2005.
146. Shahriar F, Gordon JR, Simko E: Identification of lipopolysaccharide-binding proteins in porcine milk. *Can J Vet Res* 70:243-250, 2006.
147. Mitchell TI, Coon CI, Brinckerhoff CE: Serum amyloid A (SAA3) produced by rabbit synovial fibroblasts treated with phorbol esters or interleukin 1 induces synthesis of collagenase and is neutralized with specific antiserum. *J Clin Invest* 87:1177-1185, 1991.
148. Dinarello CA: Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. *Ann N Y Acad Sci* 856:1-11, 1998.
149. Martinon F, Mayor A, Tschopp J: The inflammasomes: guardians of the body. *Annu Rev Immunol* 27:229-265, 2009.
150. Furlaneto CJ, Campa A: A novel function of serum amyloid A: a potent stimulus for the release of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-8 by human blood neutrophil. *Biochem Biophys Res Commun* 268:405-408, 2000.
151. Niemi K, Baumann MH, Kovanen PT, et al: Serum amyloid A (SAA) activates human mast cells which leads into degradation of SAA and generation of an amyloidogenic SAA fragment. *Biochim Biophys Acta* 1762:424-430, 2006.
152. Niemi K, Teirila L, Lappalainen J, et al: Serum amyloid A activates the NLRP3 inflammasome via P2X7 receptor and a cathepsin B-sensitive pathway. *J Immunol* 186:6119-6128, 2011.
153. Ather JL, Ckless K, Martin R, et al: Serum amyloid A activates the NLRP3 inflammasome and promotes Th17 allergic asthma in mice. *J Immunol* 187:64-73, 2011.

154. Song C, Hsu K, Yamen E, et al: Serum amyloid A induction of cytokines in monocytes/macrophages and lymphocytes. *Atherosclerosis* 207:374-383, 2009.
155. Olsson N, Siegbahn A, Nilsson G: Serum amyloid A induces chemotaxis of human mast cells by activating a pertussis toxin-sensitive signal transduction pathway. *Biochem Biophys Res Commun* 254:143-146, 1999.
156. Cheng N, He R, Tian J, et al: Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. *J Immunol* 181:22-26, 2008.
157. He RL, Zhou J, Hanson CZ, et al: Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. *Blood* 113:429-437, 2009.
158. Sandri S, Rodriguez D, Gomes E, et al: Is serum amyloid A an endogenous TLR4 agonist? *J Leukoc Biol* 83:1174-1180, 2008.
159. He R, Sang H, Ye RD: Serum amyloid A induces IL-8 secretion through a G protein-coupled receptor, FPRL1/LXA4R. *Blood* 101:1572-1581, 2003.
160. Lee HY, Kim MK, Park KS, et al: Serum amyloid A induces contrary immune responses via formyl peptide receptor-like 1 in human monocytes. *Mol Pharmacol* 70:241-248, 2006.
161. Su SB, Gong W, Gao JL, et al: A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J Exp Med* 189:395-402, 1999.
162. Nguyen KD, Macaubas C, Nadeau KC, et al: Serum amyloid A overrides Treg anergy via monocyte-dependent and Treg-intrinsic, SOCS3-associated pathways. *Blood* 117:3793-3798, 2011.
163. Stoneham SJ, Palmer L Fau - Cash R, Cash R Fau - Rosedale PD, et al: Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. *Equine Vet J* 33:599-603, 2001.
164. Jacobsen S, Kjølgaard-Hansen M, Petersen HH, et al: Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet J* 172:315-319, 2006.
165. Hulten C, Tulamo RM, Suominen MM, et al: A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA) -- a

- clinically useful inflammatory marker in the horse. *Vet Immunol Immunop* 68:267-281, 1999.
166. Belgrave RL, Dickey MM, Arheart KL, et al: Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *J Am Vet Med Assoc* 243:113-119, 2013.
 167. Jacobsen S, Top Adler DM, Bundgaard L, et al: The use of liquid chromatography tandem mass spectrometry to detect proteins in saliva from horses with and without systemic inflammation. *Vet J* 202:483-488, 2014.
 168. Canisso IF, Ball BA, Cray C, et al: Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. *Am J Reprod Immunol* 72:376-385, 2014.
 169. Hobo S, Niwa H, Anzai T: Evaluation of serum amyloid A and surfactant protein D in sera for identification of the clinical condition of horses with bacterial pneumonia. *J Vet Med Sci* 69:827-830, 2007.
 170. Vandenplas ML, Moore JN, Barton MH, et al: Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic. *Am J Vet Res* 66:1509-1516, 2005.
 171. Cohen ND, Chaffin MK, Vandenplas ML, et al: Study of serum amyloid A concentrations as a means of achieving early diagnosis of *Rhodococcus equi* pneumonia. *Equine Vet J* 37:212-216, 2005.
 172. Andersen UV, Reinemeyer CR, Toft N, et al: Physiologic and systemic acute phase inflammatory responses in young horses repeatedly infected with cyathostomins and *Strongylus vulgaris*. *Vet Parasitol* 201:67-74, 2014.
 173. Connolly M, Marrelli A, Blades M, et al: Acute serum amyloid A induces migration, angiogenesis, and inflammation in synovial cells in vitro and in a human rheumatoid arthritis/SCID mouse chimera model. *J Immunol* 184:6427-6437, 2010.
 174. Vallon R, Freuler F, Desta-Tsedu N, et al: Serum amyloid A (apoSAA) expression is up-regulated in rheumatoid arthritis and induces transcription of matrix metalloproteinases. *J Immunol* 166:2801-2807, 2001.

175. O'Hara R, Murphy EP, Whitehead AS, et al: Acute-phase serum amyloid A production by rheumatoid arthritis synovial tissue. *Arthritis Res* 2:142-144, 2000.
176. O'Hara R, Murphy EP, Whitehead AS, et al: Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. *Arthritis Rheum* 50:1788-1799, 2004.
177. Kumon Y, Suehiro T, Hashimoto K, et al: Local expression of acute phase serum amyloid A mRNA in rheumatoid arthritis synovial tissue and cells. *J Rheumatol* 26:785-790, 1999.
178. Koga T, Torigoshi T, Motokawa S, et al: Serum amyloid A-induced IL-6 production by rheumatoid synoviocytes. *FEBS Lett* 582:579-585, 2008.
179. Migita K, Koga T, Torigoshi T, et al: Induction of interleukin-23 p19 by serum amyloid A (SAA) in rheumatoid synoviocytes. *Clin Exp Immunol* 162:244-250, 2010.
180. Okamoto H, Katagiri Y, Kiire A, et al: Serum amyloid A activates nuclear factor-kappaB in rheumatoid synovial fibroblasts through binding to receptor of advanced glycation end-products. *J Rheumatol* 35:752-756, 2008.
181. Katz JD, Agrawal S, Velasquez M: Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Op Rheumat* 22:512-519, 2010.
182. Conde J, Scotece M, Gomez R, et al: Adipokines and osteoarthritis: novel molecules involved in the pathogenesis and progression of disease. *Arthritis* 2011:203901, 2011.
183. de Seny D, Cobraiville G, Charlier E, et al: Acute-phase serum amyloid a in osteoarthritis: regulatory mechanism and proinflammatory properties. *PLoS One* 8:e66769, 2013.
184. Kellgren JH, Lawrence JS: Radiological assessment of osteo-arthritis. *Ann Rheum Dis* 16:494-502, 1957.
185. Sukenik S, Henkin J, Zimlichman S, et al: Serum and synovial fluid levels of serum amyloid A protein and C-reactive protein in inflammatory and noninflammatory arthritis. *J Rheumatol* 15:942-945, 1988.
186. Cunnane G, Grehan S, Geoghegan S, et al: Serum amyloid A in the assessment of early inflammatory arthritis. *J Rheumatol* 27:58-63, 2000.
187. Sipe JD: Acute-phase proteins in osteoarthritis. *Semin Arthritis Rheum* 25:75-86, 1995.

188. Hrycaj P, Stratz T, Kovac C, et al: Microheterogeneity of acute phase proteins in patients with clinically active and clinically nonactive osteoarthritis. *Clin Rheumatol* 14:434-440, 1995.
189. Jacobsen S, Thomsen MH, Nanni S: Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease. *Am J Vet Res* 67:1738-1742, 2006.
190. Stack JD, Walshe K, Steele E, et al: Synovial Serum Amyloid A (SAA) Point-of-Care Test—A Valuable Aid to Immediate Diagnosis of Synovial Sepsis in Horses. *Proceeding of the Surgery Summit of the American College of Veterinary Surgeons*, San Antonio, Texas, 2014.
191. Lindegaard C, Gleerup KB, Thomsen MH, et al: Anti-inflammatory effects of intra-articular administration of morphine in horses with experimentally induced synovitis. *Am J Vet Res* 71:69-75, 2010.
192. Pepys MB, Baltz ML, Tennent GA, et al: Serum amyloid A protein (SAA) in horses: objective measurement of the acute phase response. *Equine Vet J* 21:106-109, 1989.
193. Satoh M, Fujinaga T, Okumura M, et al: Sandwich enzyme-linked immunosorbent assay for quantitative measurement of serum amyloid A protein in horses. *Am J Vet Res* 56:1286-1291, 1995.
194. Hulten C, Gronlund U, Hirvonen J, et al: Dynamics in serum of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and alpha2-globulins during induced noninfectious arthritis in the horse. *Equine Vet J* 34:699-704, 2002.
195. Christensen M, Jacobsen S, Ichiyanagi T, et al: Evaluation of an automated assay based on monoclonal anti-human serum amyloid A (SAA) antibodies for measurement of canine, feline, and equine SAA. *Vet J* 194:332-337, 2012.
196. Christensen MB, Sorensen JC, Jacobsen S, et al: Investigation of the solubility and the potentials for purification of serum amyloid A (SAA) from equine acute phase serum—a pilot study. *BMC Res Notes* 6:152, 2013.
197. Tulamo RM, Bramlage LR, Gabel AA: The influence of corticosteroids on sequential clinical and synovial fluid parameters in joints with acute infectious arthritis in the horse. *Equine Vet J* 21:332-337, 1989.

198. Trumble TN, Trotter GW, Oxford JR, et al: Synovial fluid gelatinase concentrations and matrix metalloproteinase and cytokine expression in naturally occurring joint disease in horses. *Am J Vet Res* 62:1467-1477, 2001.
199. Clegg PD, Coughlan AR, Riggs CM, et al: Matrix metalloproteinases 2 and 9 in equine synovial fluids. *Equine Vet J* 29:343-348, 1997.
200. Jacobsen S, Nielsen JV, Kjelgaard-Hansen M, et al: Acute phase response to surgery of varying intensity in horses: a preliminary study. *Vet Surg* 38:762-769, 2009.
201. McIlwraith CW, Wright IM, Nixon AJ, et al: Diagnostic and surgical arthroscopy in the horse (Third edition.), 2006.
202. McIlwraith CW, Yovich JV, Martin GS: Arthroscopic surgery for the treatment of osteochondral chip fractures in the equine carpus. *J Am Vet Med Assoc* 191:531-540, 1987.
203. Dykgraaf S, Dechant JE, Johns JL, et al: Effect of intrathecal amikacin administration and repeated centesis on digital flexor tendon sheath synovial fluid in horses. *Vet Surg* 36:57-63, 2007.
204. Anderson SL, Duke-Novakovski T, Singh B: The immune response to anesthesia: part 1. *Vet Anaesth Analg* 41:113-126, 2014.
205. Jansen NW, Roosendaal G, Wenting MJ, et al: Very rapid clearance after a joint bleed in the canine knee cannot prevent adverse effects on cartilage and synovial tissue. *Osteoarthritis Cartilage* 17:433-440, 2009.
206. Smith LJ, Marr CM, Payne RJ, et al: What is the likelihood that Thoroughbred foals treated for septic arthritis will race? *Equine Vet J* 36:452-456, 2004.
207. Neil KM, Axon JE, Begg AP, et al: Retrospective study of 108 foals with septic osteomyelitis. *Aust Vet J* 88:4-12, 2010.
208. Sanchez Teran AF, Bracamonte J, Hendrick S, et al: Evaluation of the Effect of Arthroscopic Lavage on Serum Amyloid A Concentration, Total Protein and Nucleated Cell Count in Synovial Fluid of Healthy Horses. *Proceedings of the Surgery Summit of the American College of Veterinary Surgeons, San Diego, California, 2014.*

209. Moyer W, Schumacher J, Schumacher J: Equine joint injection and regional anesthesia (5th ed). Chadds Ford, PA, Chadds Ford, PA : Academic Veterinary Solutions, LLC, 2011.

CHAPTER 8: APPENDICES AND SUPPORTING DOCUMENTATION

8.1 Data for Chapter 4

Table A1. Raw data after arthroscopic lavage

| Horse | Treatment | Time (h) | TP (g/L) | NCC ($\times 10^9$ cells/L) | Neutrophils (%) | Systemic SAA (mg/L) | Synovial SAA (mg/L) |
|-------|-------------|----------|----------|------------------------------|-----------------|---------------------|---------------------|
| H1 | Control | 0 | 22 | 0.1 | 5 | 0 | 0 |
| H2 | Control | 0 | 19 | 0.1 | 1 | 0 | 0 |
| H3 | Control | 0 | 15 | 0.2 | 9 | 0.4 | 0.1 |
| H4 | Control | 0 | 15 | 0.1 | 31 | 0.2 | 0 |
| H5 | Control | 0 | 35 | 0.1 | 14 | 2.1 | 0 |
| H6 | Control | 0 | 30 | 0.1 | 17 | 0.2 | 0 |
| H1 | Arthroscopy | 0 | 20 | 0.1 | 1 | 0.3 | 0.2 |
| H2 | Arthroscopy | 0 | 23 | 0.1 | 1 | 0.4 | 0.6 |
| H3 | Arthroscopy | 0 | 9 | 0.1 | 1 | 0.3 | 0 |
| H4 | Arthroscopy | 0 | 24 | 0.2 | 2 | 0 | 0.1 |
| H5 | Arthroscopy | 0 | 30 | 0.1 | 13 | 0.2 | 0.2 |
| H6 | Arthroscopy | 0 | 20 | 0.1 | 3 | 0.3 | 0 |
| H1 | Control | 24 | 33 | 1.8 | 52 | 0.9 | 0 |
| H2 | Control | 24 | 22 | 1.4 | 47 | 0.6 | 0 |
| H3 | Control | 24 | 18 | 0.8 | 31 | 83.6 | 0.3 |
| H4 | Control | 24 | 30 | 1.6 | 61 | 0.3 | 0 |
| H5 | Control | 24 | 28 | 1.4 | 58 | 201.3 | 2.1 |
| H6 | Control | 24 | 28 | 1.1 | 76 | 0.2 | 0 |
| H1 | Arthroscopy | 24 | 45 | 1.6 | 68 | 0.3 | 0.2 |
| H2 | Arthroscopy | 24 | 42 | 9.1 | 51 | 92.7 | 1.3 |
| H3 | Arthroscopy | 24 | 47 | 17.7 | 65 | 3.7 | 0 |
| H4 | Arthroscopy | 24 | 55 | 4.7 | 88 | 1.5 | 0.4 |
| H5 | Arthroscopy | 24 | 33 | 10.3 | 84 | 1.7 | 0.1 |
| H6 | Arthroscopy | 24 | 61 | 23.3 | 67 | 0.4 | 0 |
| H1 | Control | 48 | 38 | 1 | 7 | 0.4 | 0 |
| H2 | Control | 48 | 25 | 0.3 | 35 | 0.3 | 0 |
| H3 | Control | 48 | 20 | 1.4 | 20 | 130.8 | 1.3 |
| H4 | Control | 48 | 30 | 0.8 | 23 | 0 | 0 |
| H5 | Control | 48 | 21 | 7.1 | 55 | 243.7 | 4 |
| H6 | Control | 48 | 31 | 3.7 | 46 | 0 | 0 |
| H1 | Arthroscopy | 48 | 35 | 1.2 | 48 | 0.3 | 0 |
| H2 | Arthroscopy | 48 | 32 | 2.6 | 33 | 63.1 | 0.4 |

| | | | | | | | |
|----|-------------|-----|----|-----|----|-------|-----|
| H3 | Arthroscopy | 48 | 37 | 5.6 | 35 | 1.4 | 0 |
| H4 | Arthroscopy | 48 | 30 | 5.3 | 82 | 0.4 | 0 |
| H5 | Arthroscopy | 48 | 36 | 1.3 | 55 | 0.9 | 0 |
| H6 | Arthroscopy | 48 | 45 | 6 | 53 | 0.2 | 0 |
| H1 | Control | 72 | 32 | 1.4 | 52 | 0.2 | 0 |
| H2 | Control | 72 | 28 | 0.2 | 38 | 0.4 | 0 |
| H3 | Control | 72 | 10 | 0.9 | 15 | 61.5 | 0.8 |
| H4 | Control | 72 | 30 | 0.8 | 7 | 0 | 0 |
| H5 | Control | 72 | 20 | 3.1 | 54 | 124.6 | 1 |
| H6 | Control | 72 | 30 | 1.6 | 45 | 0 | 0 |
| H1 | Arthroscopy | 72 | 28 | 1.9 | 53 | 0.6 | 0.1 |
| H2 | Arthroscopy | 72 | 20 | 1.3 | 46 | 13.3 | 0.2 |
| H3 | Arthroscopy | 72 | 28 | 2 | 19 | 0.7 | 0 |
| H4 | Arthroscopy | 72 | 36 | 1 | 75 | 0.2 | 0 |
| H5 | Arthroscopy | 72 | 37 | 1.1 | 27 | 0 | 0 |
| H6 | Arthroscopy | 72 | 41 | 1.8 | 26 | 0.4 | 0 |
| H1 | Control | 96 | 42 | 2.1 | 51 | 0.1 | 0 |
| H2 | Control | 96 | 26 | 0.4 | 43 | 0.6 | 0.5 |
| H3 | Control | 96 | 14 | 0.7 | 15 | 3.8 | 0 |
| H4 | Control | 96 | 30 | 1.6 | 24 | 0.2 | 0.2 |
| H5 | Control | 96 | 16 | 1.3 | 47 | 17.8 | 0.3 |
| H6 | Control | 96 | 31 | 2.8 | 66 | 0.1 | 0 |
| H1 | Arthroscopy | 96 | 34 | 0.9 | 8 | 0.1 | 0.2 |
| H2 | Arthroscopy | 96 | 20 | 0.6 | 60 | 1.2 | 0 |
| H3 | Arthroscopy | 96 | 28 | 1 | 10 | 0.4 | 0 |
| H4 | Arthroscopy | 96 | 32 | 1.9 | 68 | 0.5 | 0.3 |
| H5 | Arthroscopy | 96 | 26 | 1 | 23 | 0.3 | 0 |
| H6 | Arthroscopy | 96 | 40 | 0.9 | 51 | 0.2 | 0 |
| H1 | Control | 120 | 38 | 0.8 | 14 | 0 | 0 |
| H2 | Control | 120 | 26 | 0.7 | 32 | 0.2 | 0.4 |
| H3 | Control | 120 | 22 | 0.6 | 5 | 0.4 | 0 |
| H4 | Control | 120 | 39 | 1.2 | 12 | 0.1 | 0 |
| H5 | Control | 120 | 10 | 0.8 | 3 | 1.7 | 0 |
| H6 | Control | 120 | 23 | 0.8 | 41 | 0.3 | 0 |
| H1 | Arthroscopy | 120 | 32 | 0.4 | 32 | 0.2 | 0.2 |
| H2 | Arthroscopy | 120 | 26 | 0.4 | 25 | 0.4 | 0 |
| H3 | Arthroscopy | 120 | 24 | 0.5 | 4 | 0 | 0 |
| H4 | Arthroscopy | 120 | 48 | 1.4 | 44 | 0.4 | 0.1 |
| H5 | Arthroscopy | 120 | 30 | 0.7 | 21 | 0.7 | 0 |
| H6 | Arthroscopy | 120 | 36 | 1.8 | 64 | 1.1 | 0.4 |

Table A2. Summary statistics for controls: p25, p50 (median) and p75; by time.

| Controls | | | | | |
|----------|----------|--------------------------------|-----------------|---------------------|---------------------|
| Time (h) | TP (g/L) | NCC (x10 ⁹ cells/L) | Neutrophils (%) | Systemic SAA (mg/L) | Synovial SAA (mg/L) |
| 0 | 15 | 0.1 | 5 | 0 | 0 |
| | 20.5 | 0.1 | 11.5 | 0.2 | 0 |
| | 30 | 0.1 | 17 | 0.4 | 0 |
| 24 | 22 | 1.1 | 47 | 0.3 | 0 |
| | 28 | 1.4 | 55 | 0.75 | 0 |
| | 30 | 1.6 | 61 | 83.6 | 0.3 |
| 48 | 21 | 0.8 | 20 | 0 | 0 |
| | 27.5 | 1.2 | 29 | 0.35 | 0 |
| | 31 | 3.7 | 46 | 130.8 | 1.3 |
| 72 | 20 | 0.8 | 15 | 0 | 0 |
| | 29 | 1.15 | 41.5 | 0.3 | 0 |
| | 30 | 1.6 | 52 | 61.5 | 0.8 |
| 96 | 16 | 0.7 | 24 | 0.1 | 0 |
| | 28 | 1.45 | 45 | 0.4 | 0.1 |
| | 31 | 2.1 | 51 | 3.8 | 0.3 |
| 120 | 22 | 0.7 | 5 | 0.1 | 0 |
| | 24.5 | 0.8 | 13 | 0.25 | 0 |
| | 38 | 0.8 | 32 | 0.4 | 0 |
| Total | 20 | 0.5 | 14 | 0.1 | 0 |
| | 27 | 0.85 | 31.5 | 0.3 | 0 |
| | 30.5 | 1.5 | 49 | 1.9 | 0.25 |

Table A3. Summary statistics for arthroscopic lavage group: p25, p50 (median) and p75; by time.

| Arthroscopic lavage | | | | | |
|---------------------|----------|------------------------------|-----------------|---------------------|---------------------|
| Time (h) | TP (g/L) | NCC ($\times 10^9$ cells/L) | Neutrophils (%) | Systemic SAA (mg/L) | Synovial SAA (mg/L) |
| 0 | 20 | 0.1 | 1 | 0.2 | 0 |
| | 21.5 | 0.1 | 1.5 | 0.3 | 0.15 |
| | 24 | 0.1 | 3 | 0.3 | 0.2 |
| 24 | 42 | 4.7 | 65 | 0.4 | 0 |
| | 46 | 9.7 | 67.5 | 1.6 | 0.15 |
| | 55 | 17.7 | 84 | 3.7 | 0.4 |
| 48 | 32 | 1.3 | 35 | 0.3 | 0 |
| | 35.5 | 3.95 | 50.5 | 0.65 | 0 |
| | 37 | 5.6 | 55 | 1.4 | 0 |
| 72 | 28 | 1.1 | 26 | 0.2 | 0 |
| | 32 | 1.55 | 36.5 | 0.5 | 0 |
| | 37 | 1.9 | 53 | 0.7 | 0.1 |
| 96 | 26 | 0.9 | 10 | 0.2 | 0 |
| | 30 | 0.95 | 37 | 0.35 | 0 |
| | 34 | 1 | 60 | 0.5 | 0.2 |
| 120 | 26 | 0.4 | 21 | 0.2 | 0 |
| | 31 | 0.6 | 28.5 | 0.4 | 0.05 |
| | 36 | 1.4 | 44 | 0.7 | 0.2 |
| Total | 26 | 0.55 | 16 | 0.25 | 0 |
| | 32 | 1.25 | 39.5 | 0.4 | 0 |
| | 38.5 | 2.3 | 62 | 1 | 0.2 |

8.2 Data for Chapter 5

Table A4. Raw data after repeated through-and-through joint lavage.

| Horse | Time (h) | TP (g/L) | NCC (x10 ⁹ cells/L) | Neutrophils (%) | Systemic SAA (mg/L) | Synovial SAA (mg/L) |
|-------|----------|----------|--------------------------------|-----------------|---------------------|---------------------|
| H1 | 0 | 17 | 0.1 | 1 | 0.1 | 0 |
| H2 | 0 | 25 | 0.5 | 64 | 0 | 0 |
| H3 | 0 | 10 | 0.5 | 40 | 0.6 | 0 |
| H4 | 0 | 10 | 0.5 | 58 | 0.2 | 0 |
| H5 | 0 | 15 | 0.1 | 5 | 0.2 | 0 |
| H6 | 0 | 10 | 0.1 | 3 | 0.1 | 0 |
| H1 | 24 | 20 | 2.8 | 45 | 0 | 0 |
| H2 | 24 | 28 | 8.2 | 64 | 0.1 | 0 |
| H3 | 24 | 39 | 18.6 | 57 | 0 | 0 |
| H4 | 24 | 25 | 6 | 47 | 0 | 0 |
| H5 | 24 | 37 | 19.5 | 51 | 0 | 0 |
| H6 | 24 | 60 | 38.4 | 67 | 0 | 0 |
| H1 | 48 | 15 | 1.6 | 23 | 0 | 0 |
| H2 | 48 | 30 | 2.5 | 44 | 0 | 0 |
| H3 | 48 | 35 | 6.3 | 63 | 0 | 0 |
| H4 | 48 | 25 | 2.7 | 46 | 0 | 0 |
| H5 | 48 | 26 | 5.7 | 45 | 0 | 0 |
| H6 | 48 | 35 | 11.3 | 58 | 0.2 | 0 |
| H1 | 72 | 20 | 3.5 | 58 | 0 | 0 |
| H2 | 72 | 20 | 2.9 | 59 | 0.1 | 0 |
| H3 | 72 | 31 | 2.9 | 55 | 0 | 0 |
| H4 | 72 | 22 | 11.4 | 74 | 0 | 0 |
| H5 | 72 | 26 | 6 | 31 | 0 | 0 |
| H6 | 72 | 40 | 7.9 | 64 | 0 | 0 |
| H1 | 96 | 20 | 8.6 | 75 | 0 | 0 |
| H2 | 96 | 27 | 3.6 | 59 | 0 | 0 |
| H3 | 96 | 25 | 1.8 | 37 | 0 | 0 |
| H4 | 96 | 20 | 3.5 | 56 | 0 | 0 |
| H5 | 96 | 23 | 2.3 | 21 | 0 | 0 |
| H6 | 96 | 35 | 3.2 | 47 | 0 | 0 |
| H1 | 120 | 25 | 2.7 | 52 | 0.1 | 0 |
| H2 | 120 | 29 | 2.8 | 78 | 0 | 0 |
| H3 | 120 | 26 | 3.2 | 71 | 0 | 0 |
| H4 | 120 | 25 | 14.3 | 72 | 0 | 0 |
| H5 | 120 | 29 | 6.1 | 42 | 0 | 0 |
| H6 | 120 | 37 | 6.1 | 56 | 0.2 | 0 |

Table A5. Raw data blood contamination and needle repositioning

| Horse | Blood Contamination | Needle Replacement |
|-------|---------------------|--------------------|
| H1 | 0 | 2 |
| H2 | 4 | 3 |
| H3 | 4 | 1 |
| H4 | 2 | 2 |
| H5 | 1 | 0 |
| H6 | 0 | 0 |
| H1 | 2 | 0 |
| H2 | 4 | 0 |
| H3 | 3 | 0 |
| H4 | 4 | 0 |
| H5 | 3 | 0 |
| H6 | 4 | 0 |
| H1 | 2 | 2 |
| H2 | 4 | 0 |
| H3 | 3 | 0 |
| H4 | 3 | 2 |
| H5 | 2 | 0 |
| H6 | 4 | 1 |
| H1 | 2 | 0 |
| H2 | 2 | 0 |
| H3 | 3 | 0 |
| H4 | 3 | 0 |
| H5 | 0 | 0 |
| H6 | 4 | 0 |
| H1 | 4 | 0 |
| H2 | 2 | 0 |
| H3 | 2 | 2 |
| H4 | 3 | 0 |
| H5 | 3 | 1 |
| H6 | 3 | 0 |
| H1 | 2 | 0 |
| H2 | 3 | 0 |
| H3 | 2 | 0 |
| H4 | 3 | 0 |
| H5 | 2 | 0 |
| H6 | 2 | 0 |

Table A6. Summary statistics after repeated through-and-through joint lavage: p25, p50 (median) and p75; by time.

| Time (h) | TP (g/L) | NCC (x10 ⁹ cells/L) | Neutrophils (%) | Systemic SAA (mg/L) | Synovial SAA (mg/L) |
|----------|----------|--------------------------------|-----------------|---------------------|---------------------|
| 0 | 10 | 0.1 | 3 | 0.1 | 0 |
| | 12.5 | 0.3 | 22.5 | 0.15 | 0 |
| | 17 | 0.5 | 58 | 0.2 | 0 |
| 24 | 25 | 6 | 47 | 0 | 0 |
| | 32.5 | 13.4 | 54 | 0 | 0 |
| | 39 | 19.5 | 64 | 0 | 0 |
| 48 | 25 | 2.5 | 44 | 0 | 0 |
| | 28 | 4.2 | 45.5 | 0 | 0 |
| | 35 | 6.3 | 58 | 0 | 0 |
| 72 | 20 | 2.9 | 55 | 0 | 0 |
| | 24 | 4.75 | 58.5 | 0 | 0 |
| | 31 | 7.9 | 64 | 0 | 0 |
| 96 | 20 | 2.3 | 37 | 0 | 0 |
| | 24 | 3.35 | 51.5 | 0 | 0 |
| | 27 | 3.6 | 59 | 0 | 0 |
| 120 | 25 | 2.8 | 52 | 0 | 0 |
| | 27.5 | 4.65 | 63.5 | 0 | 0 |
| | 29 | 6.1 | 72 | 0.1 | 0 |
| Total | 20 | 2.4 | 43 | 0 | 0 |
| | 25 | 3.35 | 55.5 | 0 | 0 |
| | 30.5 | 7.1 | 63.5 | 0.1 | 0 |

8.3 Animal Care Certificate of Approval for experimental study of Chapter 4

| | | |
|---|---|--|
|  UNIVERSITY OF SASKATCHEWAN | Animal Research Ethics Board (AREB) <i>Certificate of Approval</i> | |
| <hr/> | | |
| PRINCIPAL INVESTIGATOR Dr. Joe Bracamonte | DEPARTMENT/ORGANIZATION Large Animal Clinical Sciences | ANIMAL USE PROTOCOL # 20130044 |
| TITLE Evaluation of the effect of arthroscopic lavage on serum amyloid A concentration, total protein and nucleated cell count in synovial fluid of healthy horses. | | |
| SPONSORING AGENCIES University of Saskatchewan WCVN Equine Health Research Fund | | |
| BIOSAFETY NUMBER R-LAC-12 | | UNIFI FUND # |
| APPROVAL DATE: May 7, 2013 | APPROVAL OF: New Animal Use Protocol | EXPIRY DATE: April 30, 2014 |
| Full Board Meeting <input checked="" type="checkbox"/> AREB Subcommittee <input type="checkbox"/> AREB Chair and University Veterinarian <input type="checkbox"/> AREB Chair <input type="checkbox"/> | | |
| <hr/> | | |
| CERTIFICATION The University of Saskatchewan Animal Research Ethics Board reviewed the above-named research project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period. | | |
| PROTOCOL MODIFICATIONS Any modifications to this protocol must be approved by the UCACS AREB Chair prior to implementation, using the AUP Modification Form . | | |
| ONGOING REVIEW REQUIREMENTS Research programs that extend beyond one year must receive annual review. For the annual renewal, an annual review form (and progress report) must be submitted to the AREB within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the Research Ethics Office website for further instructions. | | |
| <hr/> Michael Corcoran, Chair Animal Research Ethics Board University of Saskatchewan | | <u>May 14, 2013</u> Date Issued |
| <hr/> | | |
| Please send all correspondence to: | | |
| Research Ethics Office University of Saskatchewan Box 5000 RPO University, 1607-110 Gymnasium Place Saskatoon SK S7N 4J8 Telephone: (306) 966-7928 Fax: (306) 966-2069 Email: ucacs.office@usask.ca | | |

8.4 Animal Care Certificate of Approval for experimental study of Chapter 5



UNIVERSITY OF
SASKATCHEWAN

Animal Research Ethics Board

Certificate of Approval

PRINCIPAL INVESTIGATOR

Dr. Joe Bracamonte

DEPARTMENT/ORGANIZATION

Large Animal Clinical Sciences

ANIMAL USE PROTOCOL #

20140010

TITLE

Evaluation of the effect of through-and-through joint lavage on serum amyloid A concentration, total protein and nucleated cell count in synovial fluid of healthy horses

SPONSORING AGENCIES

University of Saskatchewan - WCVN Equine Health Research Fund

BIOSAFETY NUMBER

R-LAC-12

UNIFI FUND #**APPROVAL DATE:**

March 19, 2014

APPROVAL OF:

New Animal Use Protocol

EXPIRY DATE:

March 31, 2015

Full Board Meeting ☐

AREB Subcommittee ☒

AREB Chair and
University Veterinarian ☐

AREB Chair ☐

CERTIFICATION

The University of Saskatchewan Animal Research Ethics Board reviewed the above-named research project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period.

PROTOCOL MODIFICATIONS

Any modifications to this protocol must be approved by the UCACS AREB Chair prior to implementation, using the [AUP Modification Form](#).

ONGOING REVIEW REQUIREMENTS

Research programs that extend beyond one year must receive annual review. For the annual renewal, an annual review form (and progress report) must be submitted to the AREB within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the [Research Ethics Office website](#) for further instructions.

Michael Corcoran, Chair
Animal Research Ethics Board
University of Saskatchewan

March 20, 2014
Date Issued

Please send all correspondence to:

Research Ethics Office
University of Saskatchewan
Box 5000 RPO University, 1607-110 Gymnasium Place
Saskatoon SK S7N 4J8
Telephone: (306) 966-7928 Fax: (306) 966-2069 Email: ucacs.office@usask.ca

CHAPTER 9: VITA

Dr. Andrés Sanchez Teran is originally from Quito, Ecuador. In the year of 2000 after graduating from the American School of Quito he was accepted to the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Central del Ecuador to study Veterinary Medicine. Dr. Sanchez Teran was interested in specializing in large animal and after completing his 3rd year of veterinary medicine in Ecuador, he decided to transfer to the Facultad de Ciencias Veterinarias of the Universidad Nacional del Centro de la Provincia de Buenos Aires in Argentina where he completed his Veterinary degree in 2009. During his last year of studies he was awarded a scholarship to visit the Veterinary Teaching Hospital of the University of Tennessee. There he focused on pursuing the path to become a board certified surgeon by the American College of Veterinary Surgeons (ACVS). Also, during his last two years of studies he served as a research assistant student at the Pharmacology Department where he gained a strong interest as a researcher. After graduating as a Veterinarian, he worked in two renamed Thoroughbred breeding farms in Argentina where he continued affirming his profound interest in equine surgery.

In 2010 he decided to go back to his home country, Ecuador while applying for an internship. He began a one-year Internship in Equine Surgery and Medicine at the Onderstepoort Veterinary Academic Hospital of the University of Pretoria, South Africa in March 2011. During his internship he also completed a degree of Master of Science in Veterinary Sciences with distinction and under the direct supervision of Prof. Luis Rubio-Martínez. After his internship, he was accepted at the Western College of Veterinary Medicine, University of Saskatchewan in July 2012 to complete a 3-year Large Animal Surgery Residency program together with a Master of Science degree under the direct supervision of Dr. Joe Bracamonte. This thesis serves as a partial fulfillment for the requirements to obtain the latter degree. Dr. Sanchez Teran is currently on his last weeks of clinical training as large animal surgeon and will be taking his board exams for the ACVS in February 2016.